DEC 2 6 1931

VOLUME 12 NUMBER 6

ARCHIVES OF PATHOLOGY

EDITORIAL BOARD

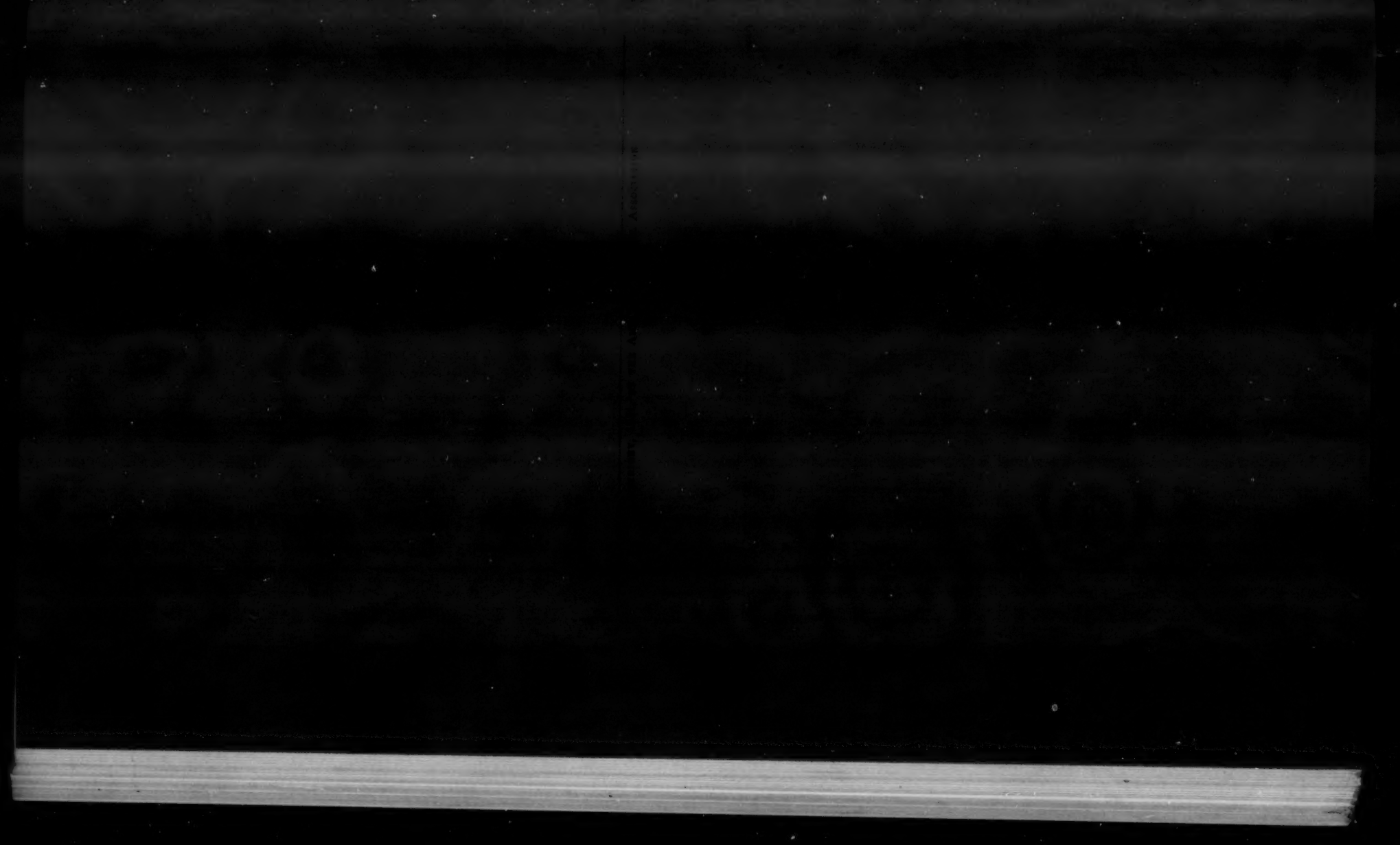
LUDVIG HEKTOEN, Chicago

JAMES EWING, New York
WILLIAM OPHÜLS, San Francisco
S. B. WOLBACH, Boston
W. G. MacCALLUM, Baltimore
ALFRED STENGEL, Philadelphia
OSCAR T. SCHULTZ, Evanston, Ill.

DECEMBER, 1931

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH DEARBORN STREET, CHICAGO, ILLINOIS. ANNUAL SUBSCRIPTION, $9.00

Entered as Second-Class Matter, Jan. 20, 1926, at the Postoffice at Chicago, Illinois, Under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in the Act of Feb. 28, 1925, authorized Jan. 27, 1926.



ARCHIVES OF PATHOLOGY

VOLUME 12 DECEMBER, 1931 NUMBER 6

HEMORRHAGE AND "SHOCK" IN TRAUMATIZED LIMBS

CHANGES IN TOTAL, FREE AND BOUND WATER OF BLOOD AND MUSCLE *

WILLIAM ROBINSON, PH.D.
AND
ELOISE PARSONS, M.D.
CHICAGO

The low blood pressure that occurs after hemorrhage and during shock is accompanied, if not directly caused, by a reduction in the volume of the circulating blood. Since blood is a liquid of which from about 78 to 81 per cent is water, it was thought that a study of the changes in the amount and condition of the water present might be another means of investigating the cause of shock. Low blood pressure was produced experimentally in barbital-anesthetized dogs by means of direct hemorrhage, injections of histamine and blows on the leg. The study of the water of the blood includes determinations of total water, and also of what are called "free" and "bound" water.

The dissolved materials and the colloids that are present in the water of the tissues have the effect of modifying some of the properties of the water in which they occur. As a consequence, the water of the tissues does not behave in all respects like water in its pure state, and it is commonly spoken of as "bound" water.

Any variations in the concentrations of substances in solution and any changes in the water-binding capacity of the colloids that take place as a result of normal physiologic or of pathologic processes may affect the degree of force with which the water is bound. Thus there is the possibility (Gortner,[1]) that a shifting takes place, either backward or forward, in the degree of water-binding, and that this may have some bearing on biologic processes.

The conception of some of the earlier workers was that part of the water in the tissues is bound and that the remainder is free; but according to more recent theories, all the water in the tissues is bound, and

* Submitted for publication, June 13, 1931.

* From the Otho S. A. Sprague Memorial Institute, the Department of Pathology and the Douglas Smith Foundation for Medical Research, University of Chicago.

1. Gortner, R. A.: Outlines of Biochemistry, New York, John Wiley & Sons, 1929.

changing conditions affect only the force with which it is bound.[2] The method employed to determine bound and free water in the present experiments, however, is applicable under either of these two conceptions.

METHODS

Determinations of Bound and Free Water.—The fact is recognized that the colloids and dissolved materials in water hold it with tenacity, resisting any force that tends to pull the water away. In the present method of making determinations a definite and constant desiccating force is used that tends to withdraw the water. The more firmly the water is bound the less, of course, can be removed. That part that can be withdrawn under a standard desiccating force is called free and the remainder, which is retained, is termed bound water under these conditions. This is an arbitrary distinction, but so long as the dehydrating force is constant this distinction serves in any series of comparative tests.

The method of Rubner,[3] termed the "heat of fusion of ice" method, is used. It consists in placing a known amount of specimen, usually about 0.5 Gm., in a prepared tinfoil container of known weight, freezing the material at a constant temperature of —20 C. for several hours, transferring the specimen and container to a calorimeter where a determination is made of the number of calories of heat required to melt the ice formed within the tissues. This determination is based on the fact that to melt 1 Gm. of ice without raising its temperature requires 80 calories of heat. By calculation, the amount of freezable water per gram of solid is determined. The final step in the process is to dry the material to constant weight for total water content. The difference between the two values indicates the amount of bound water in the specimen.

In the freezing process an efficient desiccating force is provided, since at temperatures below zero water tends to leave the colloids and dissolved materials to form ice crystals of pure water.

A detailed description of this method has been given by one of us (Dr. Robinson[4]). The process is applicable to both liquids and solids.

Care of the Dogs.—In the early experiments, the dogs were used as received from stock. Later, it was found that especial precautions had to be taken in their care and feeding because of unaccountable variations in the water content of the tissues. Each dog, therefore, was kept in a separate kennel and given food and water by the same attendant and at regular intervals. Care also was taken to avoid large changes in the temperature of the room. No animals were used until kept for one month.

Administration of the Anesthetic.—Blood drawn from dogs under ether anesthesia fluctuated so greatly in its water relations that the results could not be used. Sodium barbital was therefore used in all the experiments reported. In some of the early work, barbital was injected intravenously, but fluctuations in water content of the blood persisted. However, when the anesthetic was given by stomach tube three or four hours before the experiment, no appreciable variations in water content occurred. Approximately 0.15 Gm. per kilogram of weight in 100 cc. of

2. Briggs, D. R.: J. Phys. Chem. **35**:2914, 1931; also another article to be published in the same journal in January, 1932.

3. Rubner, M.: Abhandl. d. k. preuss. Akad. d. Wissensch., phys.-math. Kl., 1922, no. 1, p. 1.

4. Robinson, William: J. Biol. Chem. **92**:699, 1931.

water was used. At the time of the experiments, the animals were sleeping heavily and could be manipulated without being disturbed. Chart 1 shows the constancy of the water relations of the blood under these conditions during one and one-half hours. The symbols used on the charts are explained on page 5.

Control Determinations.—Control determinations were made for each dog used. Both arterial and venous blood was withdrawn under aseptic conditions from the dog one week before the experiment and under conditions closely similar to those of the experiment. Samples of blood were taken approximately every fifteen or

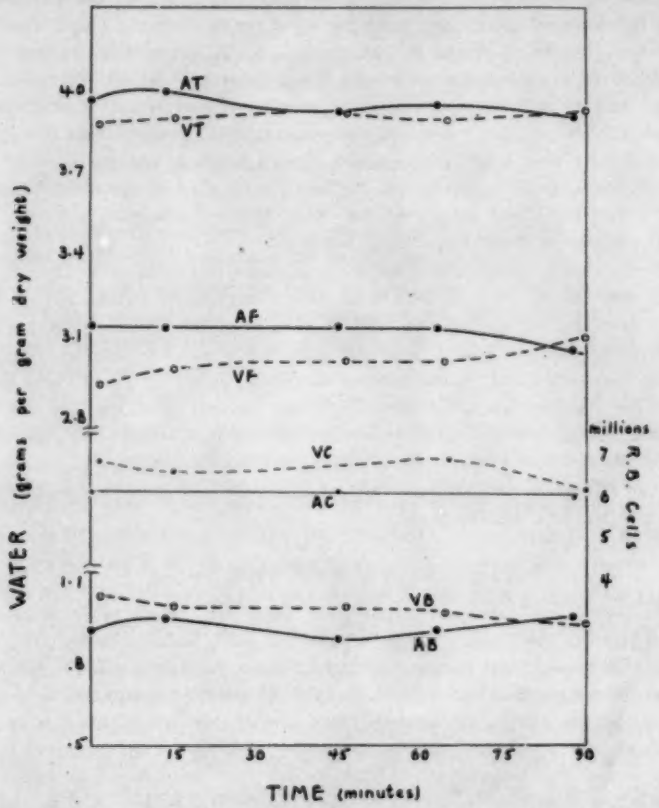


Fig. 1.—Typical control determinations of total (*T*), free (*F*) and bound water (*B*) of arterial (*A*) and venous (*V*) blood, with red cell counts (*C*). For further explanation of charts 1 to 9, see text page 5.

twenty minutes for about one and one-half hours. The dog was then given an intravenous injection of 500 cc. of 10 per cent dextrose to hasten recovery from the anesthetic. The effect of intravenous injections of dextrose on barbital-anesthetized dogs had been noted by Johnson, Lighthill and Luckhardt,[5] who found that they could not keep dogs asleep under barbital while dextrose was being administered.

5. Johnson, C. A.; Lighthill, J. A., and Luckhardt, A. B.: J. A. M. A. **95**: 576, 1930.

The blood thus drawn showed almost uniform values throughout the period. Chart 1 is typical of these control determinations that were made with each experiment.

The following week, when the dog was to be submitted to the crucial test, it was again placed under conditions closely similar to those of the experiment, and blood was again withdrawn at least twice for a second and partial control. Generally it was found that the water relations had not altered appreciably during the interval of the week. Again chart 1 is typical of all the control experiments.

Experimental Technic.—In all the experiments reported, blood was withdrawn from the left femoral artery and from the right femoral vein. These were exposed in order that the blood might be taken quickly. A cannula in the right carotid was attached to a manometer to record blood pressure. Blood was withdrawn in a syringe and at once transferred to petrolatum-coated tinfoil containers, which were then placed in glass vials and the vials corked, weighed and frozen.

When muscle was used, it was taken from a foreleg, and the exposed muscles were kept covered with hot, moist packs. For each test an entire muscle was dissected; thus the blood supply to the other muscles was least disturbed and the minimum amount of blood lost during removal.

For hemorrhage, blood was withdrawn in most instances from the left femoral artery in amounts varying from 100 to 200 cc. depending on the size of the dog.

For shock due to trauma, the left hind leg of the anesthetized animal was traumatized by blows from a 2-pound (0.9 Kg.) padded hammer, struck hard enough to bruise the soft parts without breaking bones or skin. The method is described by Parsons and Phemister.[6] When the left hind leg was traumatized, the arterial blood was collected from the left carotid artery; and as mentioned above the muscle was taken from a foreleg.

For the effects of histamine, doses of 2 cc. of 0.05 per cent histamine were injected into the left jugular vein.

THE CHARTS AND THE TABLE

Charts.—Triplicate determinations were made for every value shown on the charts. However, only the average of these is used, because of the large number of values that appear, and because deviations from the average were usually small. Deviations from the average do not necessarily indicate inaccuracies in measurement, since it should not be assumed that similar specimens taken from a tissue will all have the same total and bound water content. The extent of deviations from the mean was determined with a 10 per cent solution of gelatin in water. This was allowed to cool, small cubes were cut from the "gel" and determinations made in the usual manner. The average total water content was 8.344 Gm. per gram of dry gelatin, with a deviation of ± 0.001 Gm.; the bound water was 1.210 Gm., with a deviation of ± 0.006 Gm. It is therefore possible to obtain a fair degree of accuracy in this method.

In the charts the values for total, free and bound water are shown in grams per gram of dry weight. To find the value of water as a percentage of total weight, the number of grams of water is divided by itself plus 1 Gm. for dry weight and multiplied by 100. Thus, to convert the value 3.24 Gm. of water per gram of dry weight to a percentage basis:

$$\frac{3.24}{4.24} \times 100 = 76.4$$

6. Parsons, E., and Phemister, D. B.: Surg., Gynec. & Obst. **51**:196, 1930.

Percentage values for free and bound water are calculated on the basis of total water, and are obtained by dividing any value shown by the number of grams of total water and multiplying by 100.

The indications of the symbols used on the charts are as follows:

B P, blood pressure
A T, total water of arterial blood
V T, total water of venous blood
M T, total water of muscle
A F, free water of arterial blood
V F, free water of venous blood
M F, free water of muscle
A B, bound water of arterial blood
V B, bound water of venous blood
M B, bound water of muscle
A C, red blood cells of arterial blood
V C, red blood cells of venous blood

The values for arterial blood are shown on the solid lines, those for venous blood on the broken lines and those for muscle on the dotted lines.

Table.—The values given in the table are shown in double columns, as "grams per gram of dry weight" and as "percentages." In the percentage columns, the total water is calculated on the basis of total weight, and the free and bound water values on the basis of total water.

VARIATIONS IN WATER RELATIONS OF THE BLOOD WITH CHANGES IN CONCENTRATIONS OF RED BLOOD CELLS

A count of red blood cells was usually made each time blood was drawn. In the control period, the numbers remained fairly constant, but at the time of the crucial test they were frequently found to vary considerably. To determine what effect such fluctuations would have on the water relations of the blood the following experiment was conducted and repeated.

Freshly drawn heparinized blood was centrifugated; the plasma and corpuscles were separated, and mixtures of various concentrations of plasma and corpuscles were made as shown along the bottom of chart 2. Blood cell counts were made on mixtures containing up to 8,000,000 cells; the correlation between count and concentration is indicated by the oblique line.

The total water content of the plasma was found to be 91.6 per cent while that of the corpuscles was 66.3 per cent. In mixtures of two systems having such a wide difference in water content it would be expected that total water would be affected by changing the concentrations of the corpuscles; and this relation is shown in chart 2 by the solid line marked "total water." Variations in free water were closely similar to those of total, as appears from the broken line. The percentage of bound water in plasma was 17.6 per cent and that in the corpuscles 28.9 per cent; it will be seen that varying concentrations of corpuscles had little effect on the amount of water bound.

The two upright lines P_1 and P_2 mark off a section in which lie concentrations of red blood cells with counts from 3,500,000 to 8,000,000. Within these concentrations, the total water ranged from 86 to 76.4 per cent with a similar change in free water, while for bound water the change in values was almost negligible.

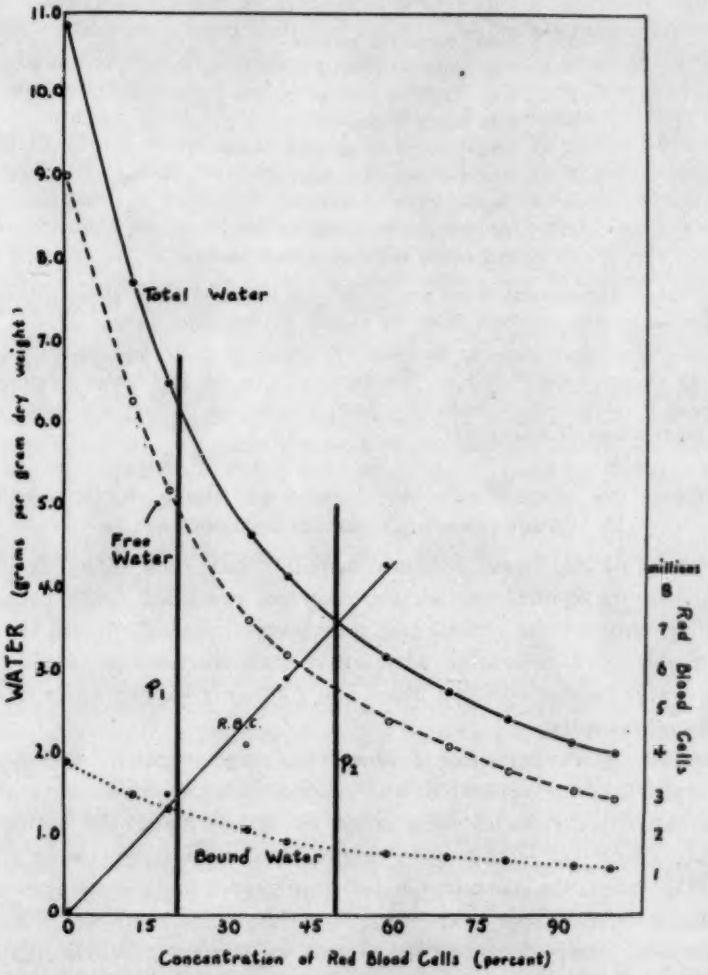


Fig. 2.—Values for total, free and bound water at various concentrations of red blood cells. The oblique line represents the correlation between the count and the concentration of red blood cells.

ALTERNATING WATER RELATIONS OF BLOOD AND MUSCLE

When specimens of muscle as well as of blood were taken, it was usually found that when a change in the total water of the blood was effected, the total water of the muscle was made to vary in the opposite

direction. These alternating values of the two tissues were found to concern largely changes in free water.

This effect is shown in charts 3, 4 and 5. It will be noted in chart 3 that when an increase in the percentage of total water of the blood

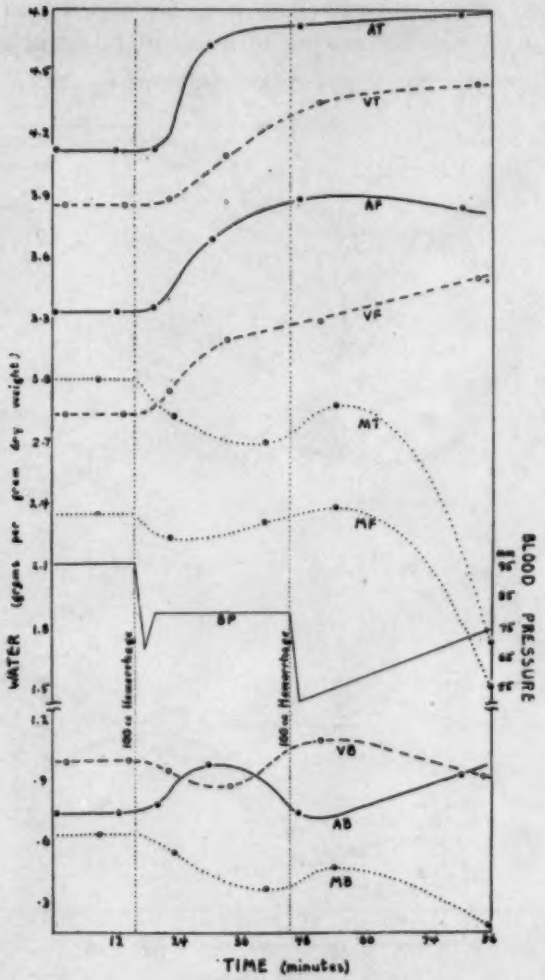


Fig. 3.—Effect of hemorrhage on the water relations of blood and muscle.

occurred, as after hemorrhage, a corresponding decrease took place in the water content of the muscle. The reverse movement is shown in chart 5; this occurred during "spontaneous" shock, when water left the blood and at the same time water entered the muscle. A similar movement followed the injection of histamine, but space does not permit the demonstration of this change in the chart.

The effect of a change in the total water of one tissue acted quickly to produce the reverse movement in the other tissue.

FLUCTUATIONS IN THE WATER CONTENT OF BLOOD AND MUSCLE, POSSIBLY DUE TO MUSCULAR ACTIVITY

In our early series, when the dogs were anesthetized on the experimental table, a considerable amount of intermittent struggling occurred

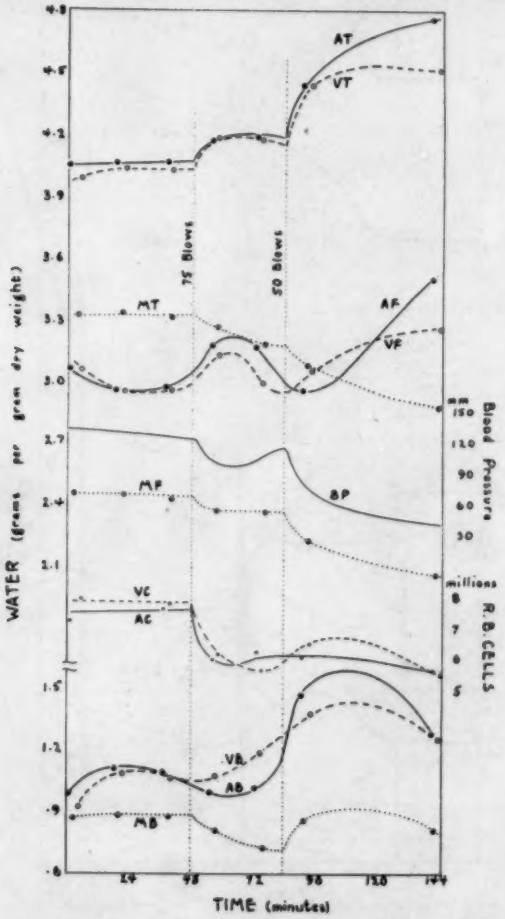


Fig. 4.—Effect of trauma to the leg on the water relations of blood and muscle.

during the experiment. Regularly, in such cases, it was noted that frequent fluctuations in the values of total, free and bound water occurred in the blood and muscles. A typical case is illustrated in chart 6, where a change of 1.2 per cent is shown to occur in the total water of the arterial blood and of 4.9 per cent in that of the venous blood. Bound water varied as much as 7.2 and 13.5 per cent, respectively.

In general, no alternation was noted here between the water content of the dissected muscle and that of the samples of the circulating blood taken at that time. It is possible that localized alternation occurs, but in our experiments this effect was probably obscured by the irregular and generalized activity.

No struggling took place, however, later, in the series, when the animals were given the anesthetic from two to three hours before the experiment began. These dogs were sleeping heavily when placed on

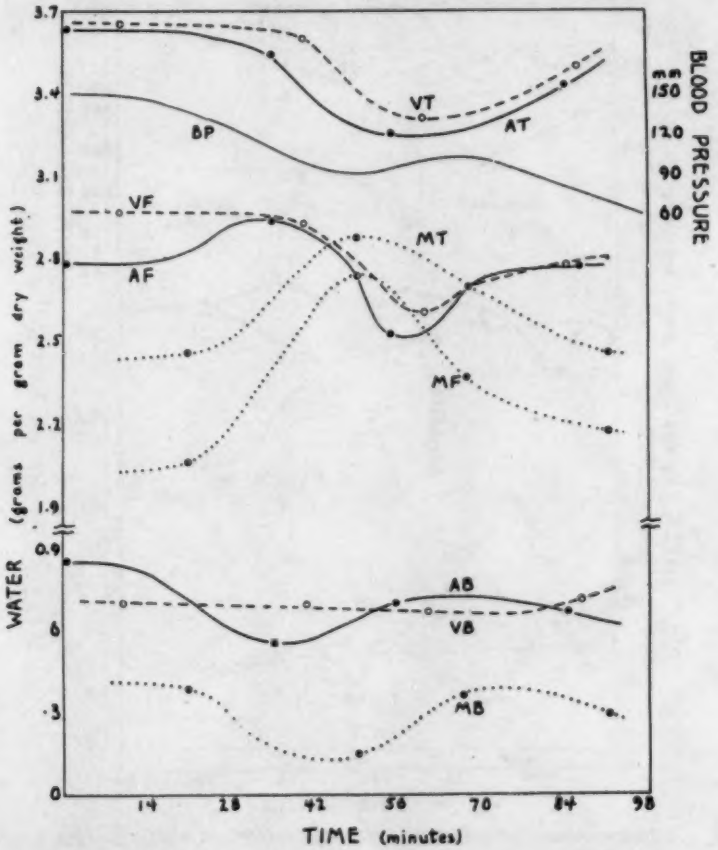


Fig. 5.—Changes in the water relations of blood and muscle during a spontaneous fall in blood pressure.

the table. In these later cases, moreover, no variations in the water content were observed; the almost uniform values obtained are shown in chart 1.

The assumption is therefore made that strenuous muscular activity may affect the water relations of the muscles and also of the blood.

EFFECTS OF HEMORRHAGE

The effects of hemorrhage were observed on six controlled animals. Blood was withdrawn usually twice, several minutes apart, and in amounts ranging from 100 to 200 cc., according to the size of the dog. The percentage of total water of both arterial and venous blood increased regularly after the first, and sometimes following the second, withdrawal. This phenomenon is shown in charts 3 and 7.

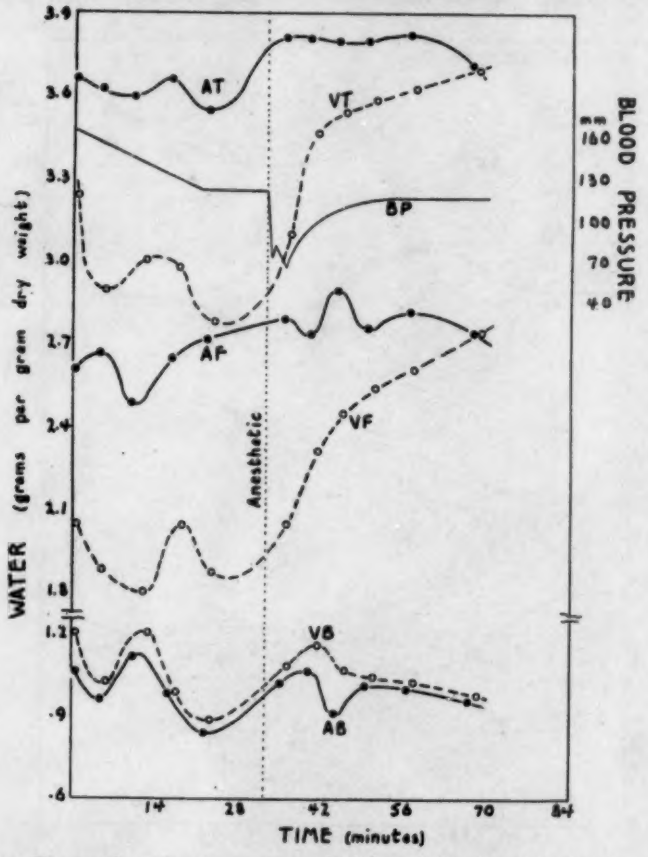


Fig. 6.—Fluctuations in the water relations of blood evidently the result of muscular activity.

In the experiment illustrated in chart 7, in which a dog weighing 11 Kg. was used, increases in total, free and bound water in the blood were shown to take place after each hemorrhage. The total water of both arterial and venous blood rose from 4.1 to 5 Gm. per gram of dry weight, which is an increase from 80.2 to 83.4 per cent. The free and bound water increased in smaller amounts, their sum in grams per gram of dry weight at any point being equal, of course, to the value shown for total water.

Specimens of muscle in addition to blood were taken in the experiment illustrated in chart 3, in which a dog weighing 12 Kg. was used. The percentage of total water is shown to increase in the blood following hemorrhage, but at different rates in the arterial and venous blood. Similar changes are seen for free water, while the bound water values for arterial and venous blood vary alternately.

With muscle, the effect of hemorrhage was evident in a general fall in total, free and bound water. The total water content fell from 3 to

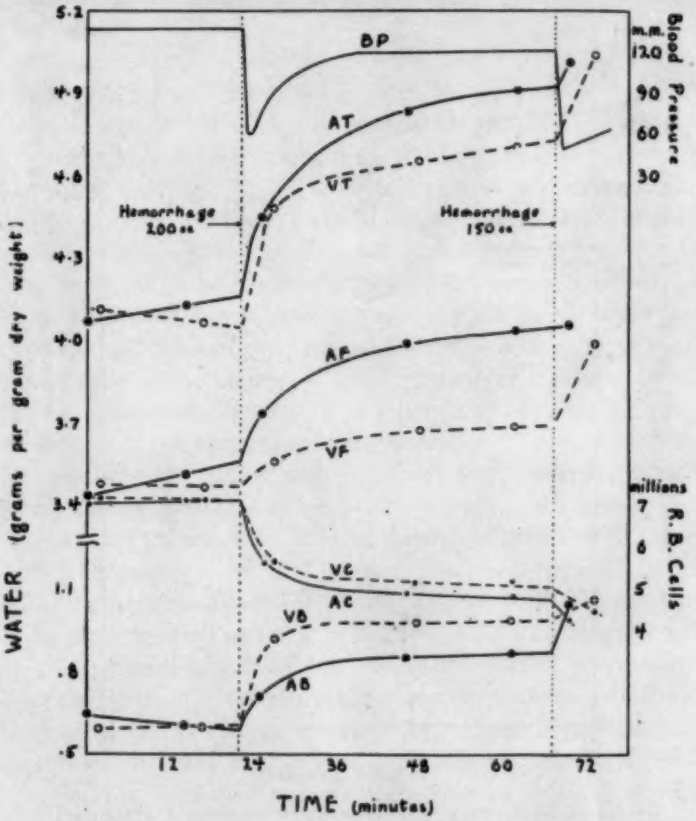


Fig. 7.—Effect of hemorrhage on the water relations of blood.

1.7 Gm. per gram of dry weight, or from 75 to 63.2 per cent, being a loss of 11.8 per cent. This is a commonly observed phenomenon, namely, that hemorrhage causes a desiccation of the tissues other than blood, the water of the tissues, especially of the skeletal muscles, passing into the blood to replace its lost volume. The passage of water from the skeletal muscles to the blood was, in this instance, largely a transfer of free water; but at the same time, the bound water fell in a similar manner, though in a smaller proportion.

Blood pressure fell from 96 mm. of mercury to 67 and 50 mm., respectively, after the first and second withdrawals, to regain a pressure of 74 mm. forty minutes later.

EFFECTS OF THE INJECTION OF HISTAMINE

These tests were made for comparison of the effects with those resulting from traumatism of a limb. In this series, four dogs were

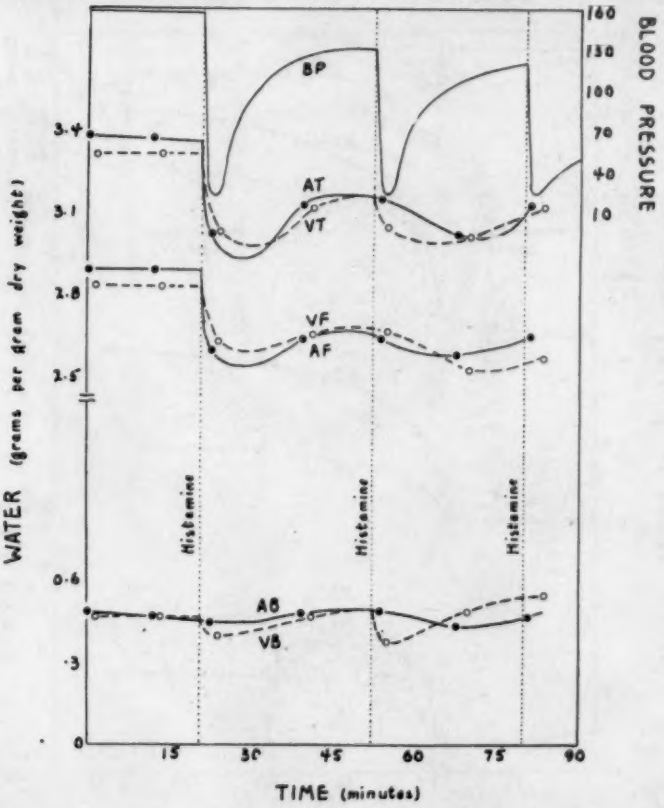


Fig. 8.—Effect of injection of histamine on the water relations of blood.

used. The changes typically found are shown in chart 8. Quickly following the first injection of histamine, the total water content of both arterial and venous blood fell from 3.3 to 3 Gm. per gram of dry weight, which was a decrease of about 2 per cent of water. A partial recovery took place, and at the time of the second injection another, but less marked, fall occurred. These fluctuations were largely changes in free water. Blood pressure fell in a characteristic manner after each injection and was followed by a slow and incomplete recovery, which always failed to reach the normal level.

The red blood counts typically rose from a normal value of about 5,760,000 to about 6,080,000 after the first injection, and increased after the second and third injections until a count as high as 8,400,000 had been reached. This, of course, is to be expected after the marked movement of water from the blood to the tissues that follows the injection of histamine.

In all cases in which specimens of muscle were taken for analysis of water content before and after injection of histamine, it was found that the muscle gained at about the same rate that the blood lost water.

EFFECTS OF TRAUMA

The six dogs used in this series may be separated into two groups according to the initial effect of trauma on the water content of the blood. The effect in the first group, which consisted of four dogs, is represented by chart 9. This was an experiment on a normal dog weighing 14 Kg. After the first series of blows, the total water content of both arterial and venous blood gradually fell from 3.5 to 3.2 Gm. per gram of dry weight, which was a loss of 1.5 per cent of total weight. However, following the second trauma, the outgo of water from the blood ceased, and a rapid rise began. During the next thirty minutes, in which a third series of blows was given, the water rose to approximately 4 Gm., an immediate increase of 3.5 per cent of total weight, and a net increase of 2 per cent over the initial value.

This movement of water was usually of free water, the bound water remaining almost constant, although in one animal the bound water rose after the second trauma from 0.35 to 1 Gm. per gram of dry weight, an increase of 9.3 per cent of total water. The blood pressure fell slightly following the first trauma and while the water was leaving the blood.

In the experiment illustrated in chart 4, the blood pressure fell about 20 mm., but regained most of this before the second trauma. A much greater fall occurred after the second and third series of blows, when the pressure dropped from about 144 to 30 mm. of mercury in about thirty minutes. Counts of red blood cells were not obtained in all cases. When taken, they indicated a slight concentration at the time of the first trauma coincident with the loss of water and fall in blood pressure. Counts also showed a dilution when the blood regained water, as is seen under conditions of hemorrhage.

During the period of trauma, which lasted for from one to three hours, the traumatized leg always became markedly discolored and swollen. This phenomenon was also noted by Parsons and Phemister [6] in a large series of experiments on trauma to the leg, and they observed that the swelling was accompanied by a progressive fall in blood pressure. They weighed both the traumatized and the normal leg and found that

the injured one was much the heavier. On dissection, they found the tissues of the traumatized leg to be edematous and engorged with blood.

Further effects of severe trauma were shown in the second group, which consisted of two dogs; here a rise instead of a fall occurred in the total water of the blood following the first series of blows. This is shown in chart 4, the animal used weighing 21.4 Kg. The increases of 2.5 per cent that took place in the total water of the arterial blood and

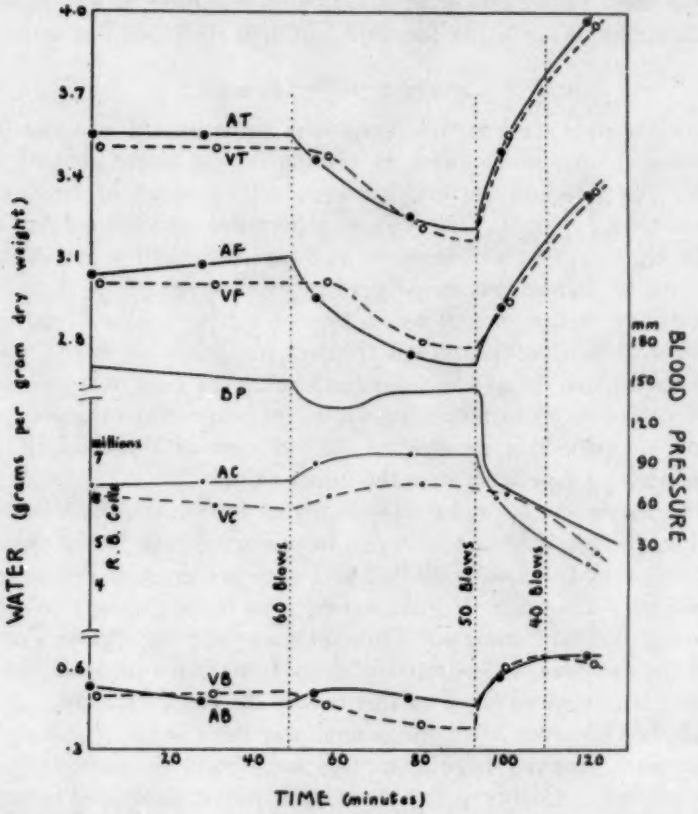


Fig. 9.—Effect of trauma to the leg on the water relations of the blood.

of 2 per cent in that of the venous blood at the termination of the test were similar to those seen in the first group of dogs. Bound water increased 8.7 per cent in the arterial and 4.4 per cent in the venous blood after the second series of blows, but fell somewhat at the end.

Specimens of muscle were taken in this experiment, and as usual were cut from the left foreleg, the trauma being administered to the right hind leg. The alternating values of the water contents of the blood and muscles noted in a previous section as a general phenomenon are seen also in this chart, which shows that as the proportion of water

of the blood rose that of the water of the muscles fell. The change was largely in the free water content.

Blood pressure fell slightly after the first trauma, recovered almost completely and fell again following the second series of blows from nearly 120 to about 40 mm. of mercury. The number of red blood cells decreased in the manner usually noted following hemorrhage.

OTHER "SHOCKLIKE" CONDITIONS

A case of "spontaneous" fall in blood pressure is included to show the changes in the water relations that ensued. The experiment is

Maximum Changes That Occur in the Water Relations of Blood and Muscle Following Hemorrhage, Injection of Histamine and Trauma to the Leg

	Total Water		Free Water		Bound Water		
	Gm. per Gm. Dry Weight	Percentage of Total Weight	Gm. per Gm. Dry Weight	Percentage of Total Water	Gm. per Gm. Dry Weight	Percentage of Total Water	Comment
Normal arterial blood	4.11	80.4	3.37	82.0	0.74	18.0	
1 hr. after hemorrhage	4.78	82.7	3.99	84.4	0.92	19.2	
Normal venous blood	3.86	79.4	2.87	74.4	0.99	25.6	
1 hr. after hemorrhage	4.44	81.6	3.53	79.5	0.91	20.5	See chart 3
Normal muscle from foreleg	3.00	75.0	2.37	79.0	0.63	21.0	
1 hr. after hemorrhage	1.77	63.2	1.54	89.5	0.18	10.5	
Normal arterial blood after injection of histamine	3.38	77.2	2.89	85.5	0.49	14.5	
	3.01	75.1	2.57	85.4	0.44	14.6	See chart 8
Normal venous blood after injection of histamine	3.32	76.8	2.85	85.9	0.47	14.1	
	3.01	75.1	2.52	83.7	0.49	16.3	
Normal arterial blood	4.05	80.2	3.06	75.6	0.99	24.4	
1½ hr. after trauma	4.77	82.7	3.49	73.2	1.28	26.8	
Normal venous blood	4.04	80.1	2.95	73.0	1.09	27.0	
1½ hr. after trauma	4.53	81.9	3.26	72.1	1.26	27.9	See chart 4
Normal muscle	2.45	71.0	1.58	64.5	0.87	35.5	
1½ hr. after trauma	2.07	67.4	1.26	60.9	0.81	39.1	

represented in chart 5. When the animal had been anesthetized, and specimens of blood and muscle were being taken, a steady fall in blood pressure, from 150 mm. to 120 mm. of mercury was noted. The decrease continued for forty-five minutes; at that time it had reached 92 mm. A slight recovery occurred, but terminated in a fall to 60 mm. During the first fall in blood pressure, the total water of both arterial and venous blood decreased from 78.4 to 76.8 per cent, while the water of the muscle increased from 71.1 to 74.2 per cent, indicating that a loss of liquid from the blood to the fixed tissues was involved in the fall of blood pressure. In about fifty-five minutes, the blood began to gain and the muscles to lose water, associated with which occurred a temporary rise in blood pressure. Some fluctuations in the bound water occurred during the period, but the changes were mostly in the free water.

The cause of the circulatory failure in this animal is not obvious. It occurred in one of the earlier experiments in which the sodium barbital was injected intravenously, a preliminary dose of morphine having been administered hypodermically. In this dog, the muscle for analysis was removed from the abdominal wall instead of from the foreleg, and the peritoneum was injured, in one place being torn so that the intestines extruded.

While the question of circulatory failure resulting from abdominal operation is not within the scope of this report, the changes shown in this experiment were similar to those noted in so-called surgical shock, and showed that when the water left the blood, it entered the muscle.

COMMENT

In these experiments, the circulatory embarrassment or failure that occurs after hemorrhage, trauma to an extremity or injection of histamine was followed by quantitative determinations of the total, free and bound water in the circulating blood and skeletal muscles. This method of studying water relations is reported as an adjunct to the investigation of the physiologic and pathologic changes involving blood volume, edema and dehydration.

Previous to this study, the method had not been employed over a period of time, with changing conditions in the blood or other tissues; consequently, no other results are available for comparison. Values for total water content alone evidently do not give an adequate picture of the changes that occur. Such data, however, may have more significance when accompanied by determinations of the degree with which the water is bound by the colloids and dissolved substances present.

In the control tests, when blood was withdrawn from the dogs, without disturbing them, during a period of from one and a half to two hours, it was found that the total, free and bound water of the blood and muscles remained practically constant throughout the period. In addition, similar values were found one week later, when the animals were again tested. This uniformity was observed over a period of a week in numerous cases, and leads to the conclusion that it is a normal condition. Any changes in water content, therefore, such as those that are demonstrated to follow hemorrhage, trauma and injection of histamine, appear to have some significance, although the observed changes are usually not more than from 3 to 5 per cent.

Changes in the water content of blood or muscle, which may appear to be small when calculated on a percentage basis, may nevertheless represent a proportionately large volume of water. For instance, in a 10 Kg. dog, the muscle of which, containing 75 per cent water, makes up 45 per cent of the total weight of the dog, a change of 5 per cent in

the water content of the muscle would involve a movement of 225 cc. of water. In the case of the blood, containing 80 per cent water and making up about 7.7 per cent of the total weight of the dog, a change of 5 per cent in the water content would mean a change in volume of 38.5 cc.

The relative proportions of water in the muscle and in the blood are approximately as 225 is to 38. Therefore if, say, 5.9 per cent of the water of the blood should move to the muscle, it would raise the water content of the muscle by only 1 per cent.

A comparison of values for free and bound water in tissues obtained by different investigators seems to be of limited worth, because the values are derived by methods that have not been standardized. The various methods employed at present in the study of bound water have recently been discussed by Briggs.[7] However, it is noted here that Hill,[8] using a vapor pressure method, found about 97 per cent of the total water of the blood to be free water, in that it could act as a solvent for salts; he found the same percentage of free water in the muscles. In the determinations reported here, the amount of free or freezable water at — 20 C. varies in the control animals from 65 to 85 per cent.

Compensation for a nonfatal hemorrhage soon sets in; the plasma in the blood is renewed, and the blood regains its normal volume within a few hours in slight hemorrhage. The red blood corpuscles and the hemoglobin, however, are restored more slowly, requiring a number of days or several weeks. The rapidity with which the plasma is restored is shown by the increase in the total water content of the blood. The increase is largely in freezable water, but there is also an appreciable rise in bound water. The water, in large part, comes from the muscles, as shown by a decrease in the water present there. The rate at which the interchange of fluids takes place indicates that the water is loosely bound in a physical sense, and that the walls of the blood vessels are easily permeable.

The action of histamine appears to be on the capillary wall, which Krogh[9] claimed to be actively contractile and to have an intrinsic tone that can be modified by nervous and chemical influences. The dilation of the capillaries produces a stasis in the capillary bed and permits extravasation of the plasma. The circulating blood shows a concentration of corpuscles and a decrease in water content, the low blood volume producing the fall in blood pressure. Dale,[10] one of the discoverers and first investigators of histamine, pointed out the resemblance of certain states of traumatic shock to the effects of injection of histamine.

---

7. Briggs (footnote 2).

8. Hill, A. V.: Proc. Roy. Soc. London, s. B. **106**:477, 1930.

9. Krogh, A.: The Anatomy and Physiology of Capillaries, New Haven, Conn., Yale University Press, 1924.

10. Dale, H. H.: Bull. Johns Hopkins Hosp. **30**:257, 1920.

"Traumatic" shock (so-called because it is a secondary shock that usually develops slowly following an injury with little or no obvious hemorrhage) has been the subject of much investigation. The studies of the Medical Research Committee during the Great War were summarized in a monograph by Cannon.[11] The experimental method that produced a low blood pressure and other phenomena most nearly similar to the condition seen in the injured soldier consisted in traumatizing one of the posterior extremities of an animal. The theory that the committee thought satisfied the findings was that of traumatic toxemia. This presumes the formation of a toxic substance in the bruised or injured tissue, which enters the blood stream to produce a capillary dilatation and the resultant lowering of blood pressure. Since histamine is a toxic substance that acts in this manner when injected into the blood, the toxic agent was thought to be histamine-like.

Recently the theory of traumatic toxemia has been questioned, and further investigations have failed to prove the presence of a toxic agent either in the circulating blood or in the traumatized tissue of an animal the extremity of which has been injured. Parsons and Phemister[6] repeated the experiments of Bayliss and Cannon on a large number of dogs and found that traumatism of the limb is accompanied by a general anemia and a corresponding increase in the limb volume, which is largely due to hemorrhage into the damaged tissues. They found no evidence of a toxic, blood pressure-lowering substance, and considered it preferable to speak of hemorrhage or of shock due to hemorrhage, rather than to use the term "shock" when acute loss of blood in open or closed wounds is the cause of marked circulatory failure or embarrassment.

Blalock,[12] using the same experimental method, found that blood pressure could not be reduced to the shock level by trauma to a posterior extremity without causing the loss of a sufficient part of the blood volume into the traumatized area to account for the decline in pressure. He found a greater proportionate loss in plasma than in red cells, which accounted for the concentration of the blood elsewhere. The important demonstration by Keith and others[13] of the reduction in the amount of the circulating blood in secondary shock has not been sufficiently emphasized in explaining the condition, although it has been used as a basis for the treatment of persons suffering from secondary shock.

---

11. Cannon, W. B.: Traumatic Shock, New York, D. Appleton and Company, 1923.

12. Blalock, Alfred: Arch. Surg. **20**:959, 1930.

13. Keith, N. B.: Medical Research Committee, Reports of the Special Investigation Committee on Surgical Shock and Allied Conditions: IX. Blood Volume Changes in Wound Shock and Primary Hemorrhage, London, His Majesty's Stationery Office, 1919, Special Report Series, no. 27.

In comparing the changes in the amount of water in the blood following trauma to the leg with the changes after injection of histamine, it is seen that in a number of animals there is a similarity, in that an initial decrease takes place in the water in the circulating blood. This is explained in the case of the injection of histamine on the basis of capillary dilatation with increased capillary permeability.

In the case of injury to the leg there is a localized erythema with capillary dilatation immediately following the hammering of the leg. It is assumed that a loss in plasma takes place before the extravasation of erythrocytes occurs. Actual hemorrhage into the swollen tissues of the leg is a slower process. In our experiments, the effect was hastened by a second trauma, after which occurred a marked swelling of the leg and a fall in blood pressure. The circulating blood became diluted, the muscles lost water, and the result was a more watery blood—a condition that exists after hemorrhage. In the animals in which the injury to the leg was followed immediately by an increase in the water content of the circulating blood, the blows of the hammer were probably sufficient actually to rupture vessels, so that bleeding occurred rapidly into the tissues of the leg, producing the characteristic water changes after hemorrhage.

CONCLUSIONS

A study has been made of the water content of the blood and muscles in respect both to total water and to water in the "free" and in the "bound" state, in animals under the influence of hemorrhage, histamine and trauma. The observations show that with hemorrhage there is a compensatory transfer of water chiefly in the "free" state from the muscles to the blood. In "histamine shock," the fall of blood pressure is associated with, and perhaps is partly the result of, a transfer of water from the blood to the muscles. In "shock" produced by severe traumatism to a leg, the evidence indicates that with minor trauma there is in some cases a loss of fluid from the blood to the muscles associated with a fall in blood pressure, and that with major trauma the picture is that of hemorrhage, in that there is a dilution of the blood remaining in the general circulation with water withdrawn from the muscle. These observations are in support of the work of Parsons and Phemister and others which indicates the importance of the loss of blood in traumatized tissue in causing the fall of blood pressure noted after severe traumatism.

SUMMARY

In hemorrhage, injection of histamine and shock due to hemorrhage, an alternation exists between the water content of the blood and that of the skeletal muscles. That is, under these conditions, when an increase occurs in the water of the blood, a decrease takes place in the

water of the muscles, and vice versa. The changes are almost simultaneous, and in some cases seem to be proportionate.

Generalized muscular activity produces slight but frequent fluctuations in the water content of both blood and muscles, sometimes increasing and at other times decreasing the percentage of water present. The alternation noted in the preceding paragraph was not observed under these conditions.

A hemorrhage of from 200 to 300 cc. of blood in dogs weighing from 10 to 14 Kg. produces an increase in the percentages of the total and free water contents of the blood. That this indicates a dilution of the blood is shown by the decrease in the erythrocyte count. A fall in the total, free and bound water in the muscles accompanies the rise in the water of the blood.

The sudden fall in blood pressure that follows the injection of histamine is accompanied by a decrease in the total and free water of the blood and small changes in the bound water. The circulating blood becomes concentrated, as shown by both the decrease in water content and the increase in the erythrocyte count. With the loss of water from the blood occurs a rise in the water of the muscle.

In trauma to the leg, in some dogs, the water in the blood is decreased after the first trauma, owing to passage of water from the blood to the general skeletal muscles, and is increased following a second trauma, probably because of the rupture of blood vessels and consequent internal hemorrhage. In other dogs, no preliminary concentration of the blood occurs, but a dilution as is seen in hemorrhage. In all dogs, after the traumatized leg has become markedly swollen, there occur an increase in the water of the blood, a decrease in that of the muscles distant from the trauma, and a decrease in the number of circulating erythrocytes, indicating hemorrhage in the limb. The condition at the end of the experimental trauma shows water relations similar to those that follow hemorrhage.

NONSYPHILITIC AORTIC VALVE DEFORMITY*

B. J. CLAWSON, M.D.
MINNEAPOLIS

There are two kinds of nonsyphilitic diseased aortic valves: those with active vegetations and those that are deformed from thickening and roughening due to scar tissue and, in many cases, to calcium deposits.

The term valve defect was used by Libman [1] and others to denote a healed condition of the valve in contrast to active inflammation of the cusps as seen in acute rheumatic or bacterial endocarditis.

Healed aortic valve deformities are sometimes incorrectly diagnosed. Not infrequently the stigma of syphilis is unfairly attached to a person who has an aortic valve deformity, nonsyphilitic in origin. Both clinicians and pathologists make diagnoses of arteriosclerotic valve deformity. The concept of an arteriosclerotic aortic valve deformity depends, in general, on the work of Mönckeberg [2] who, after studying aortic valves in which there was extensive calcification, decided that the process bringing about the change in the cusps was the same as that found in the intima of the aorta in senile arteriosclerosis. Mönckeberg held the opinion that the arteriosclerotic process extended from the aorta to the valves by way of the sinus of Valsalva. His observations suggested that the change in the cusps was most pronounced at the angle where the cusps and the aorta join. Margolis, Ziellessen and Barnes [3] concluded that the etiology and pathogenesis of this type of valve lesion could not be determined with certainty, but that clinical and anatomic data indicated that in some cases the lesion may have an inflammatory basis, whereas in others it may be the result of a noninflammatory, degenerative process.

The frequency of cases coming to autopsy with a healed calcified nodular aortic valve lesion and the diversity of opinions among internists and pathologists concerning the frequency, etiology and pathogenesis of these lesions led to a study of healed aortic valve deformities

* Submitted for publication, July 9, 1931.

* From the Department of Pathology, University of Minnesota.

1. Libman, E.: The Clinical Significance and Course of Subacute Bacterial Endocarditis, Brit. M. J. **2**:30, 1920.

2. Mönckeberg, J. G.: Der normale histologische Bau und die Sclerose der Aortenklappen, Virchows Arch. f. path. Anat. **176**:472, 1904.

3. Margolis, H. M.; Ziellessen, F. O., and Barnes, A. R.: Calcareous Aortic Valvular Disease, Am. Heart J. **6**:349, 1931.

in autopsy material of the department of pathology at the University of Minnesota. In this study special emphasis was placed on the so-called arteriosclerotic valve as described by Mönckeberg.[2] This type of aortic valve deformity was called the calcified nodular type by Clawson and Bell in a former paper.[4]

An effort is made in this work to see if arteriosclerosis of the valve is ever of a degree severe enough to be responsible for cardiac failure, and to determine, if possible, the etiology and pathogenesis of calcified nodular aortic valve deformity. This type of lesion will be spoken of as the calcified nodular aortic valve deformity, to distinguish it from the syphilitic aortic valve deformity and from the healed non-calcified rheumatic lesions of the aortic valve.

The calcified nodular type of aortic valve deformity deserves consideration from two angles: (1) the frequency of the lesion with

TABLE 1.—*Degree of Stenosis in Sixty-Eight Cases of Calcified Nodular Aortic Valve Deformity*

Degree of Stenosis	Cases	
	Number	Per Cent
0	6	9.0
+	17	25.0
++	16	23.5
+++	29	42.5
Total	68	100

Grade + is the least stenosis which can be detected anatomically. Grade +++ is the most severe stenosis; with such a degree the aortic orifice is almost closed. Grade ++ is intermediate between grades + and +++.

aortic insufficiency or stenosis and (2) the etiology of the lesion. The former consideration is of much significance to the internists, and the latter chiefly concerns the pathologists.

FREQUENCY

Aortic insufficiency is generally admitted to be a not infrequent condition. Aortic stenosis has been looked on as a condition that seldom occurs. Osler [5] referred to it as rare. My findings are not in agreement with this idea.

In the autopsy material that I studied there were represented 253 deaths due to lesions of the cardiac valves, and in 161 (63.6 per cent) of the cases there was aortic valve deformity, either alone or in association with deformities in other valves. Ninety-three (57.7 per cent) of the cases in which there was aortic valve deformity were of the Mönckeberg

4. Clawson, B. J., and Bell, E. T.: Valvular Diseases of the Heart, Am. J. Path. **2**:192, 1926.

5. Osler, W.: The Principles and Practice of Medicine, New York, D. Appleton and Company, 1925, p. 831.

type, or what I have called the calcified nodular type. In 90 per cent of the cases in which there was aortic valve deformity alone, it was of the calcified nodular type. In the cases in which the aortic valve deformity was severe, the calcified nodular type was the one generally found.

The frequency of a relatively marked grade of stenosis was high. Table 1 shows the degree of stenosis found in 68 cases examined from the 93 cases presenting calcified nodular valve deformities.

It is seen in these 68 cases of calcified nodular aortic valve deformity that some degree of stenosis is present in 62 (91 per cent). Stenosis of grades ++ and +++ can generally be detected by physical signs (based on the histories of the cases). It appears that about 66 per cent of the cases of calcified nodular aortic valve deformity show aortic stenosis clinically. It becomes evident from the foregoing figures that at least 38 per cent of all nonsyphilitic aortic valve deformities or 24 per cent of all nonsyphilitic old valve defects will show stenosis clinically. This frequency is much higher than is generally believed. The frequency of stenosis with aortic valve deformity may even be greater than 38 per cent, since it is possible to have an aortic stenosis with noncalcified aortic valve deformity. This, however, in my experience, is not common, since a nonsyphilitic aortic valve deformity of marked severity usually becomes calcified. It should be borne in mind that aortic stenosis is common. This is of significance to the internist in making a diagnosis of the type of valve injury in a case in which death is due to valve deformity. Regurgitation is commonly associated with the stenosis, but stenosis occurring alone is frequent.

ETIOLOGY

There has been much discussion concerning the origin and pathogenesis of the calcified nodular aortic valve deformities. Some observers believe that the thickened, calcified aortic cusps are the end-result of repeated attacks of rheumatic valvulitis with scar formation and calcification similar to that in inflammatory scar tissue in other valves and in other parts of the body. Others look on the aortic lesions as a pure metabolic process. Mönckeberg [2] considered the changes in the valves a part of the phenomenon of arteriosclerosis. Libman also described the lesions as arteriosclerotic in origin. The lesions in the 93 cases concerned here are studied from both the infectious and the metabolic angles to see which of the two factors seems more likely to be the etiologic agent. In attempting to arrive at the probable etiologic basis, important data can be collected from the histories of the cases, such as age and a history of acute rheumatic fever, and from the autopsies, such data as evidence of pericarditis, the condition of the arch of the aorta, frequency of defects of other valves and the gross and microscopic structures of the valves themselves.

Those who believe that these calcified nodular valve deformities are due to faulty metabolism, which results in calcification of the thickened cusps independent of an associated inflammation, stress the fact that this condition in the aortic valve is most common in older people. It is therefore assumed that these lesions are part of a senile process.

In order to study the relation of calcified nodular aortic valve deformities to age, a comparison of the age incidence of the calcified nodular aortic deformity is made with the age incidence of valve deformities that did not present the calcified nodules in the aortic cusps. By referring to figure 1 it is seen that death occurred in the group

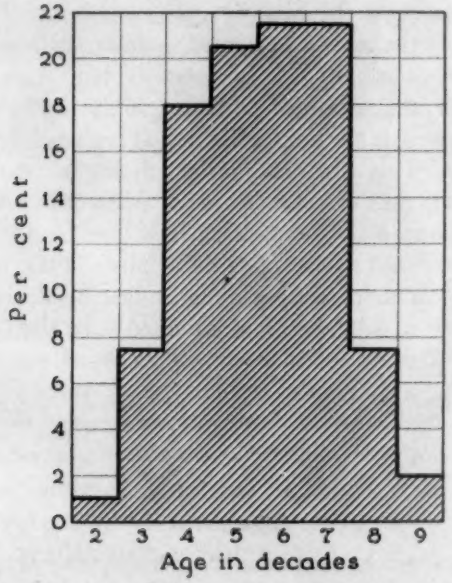


Fig. 1.—Age incidence of calcified nodular aortic valve deformity (93 cases).

with the calcified nodular aortic valve deformities most frequently in the sixth and seventh decades (43 per cent). In only 26 per cent of the group did death occur in the second, third and fourth decades, while in 74 per cent it occurred in decades 5 to 9. Yet it is to be observed that 47 per cent of the persons in this group died by the time they were 50 years old. The disease, obviously, cannot be considered entirely a disease of old age.

Figure 2 shows the ages at which death occurred most frequently in 160 persons not having calcified nodular aortic valve deformities. The greatest number of deaths occurred in the fifth decade, or about 15 years earlier than in the group with calcified nodular aortic valve deformities. Forty-four per cent of the group without the calcified

aortic valves died in decades 1 to 4, while 56 per cent died in decades 5 to 8. It is evident that death occurs at an older age in the group with calcified nodular aortic valve deformities than in the group in which such aortic deformities are not present. The question arises whether senile disease is the deciding factor in the pathogenesis of the valve deformities in this group or whether persons in this group live

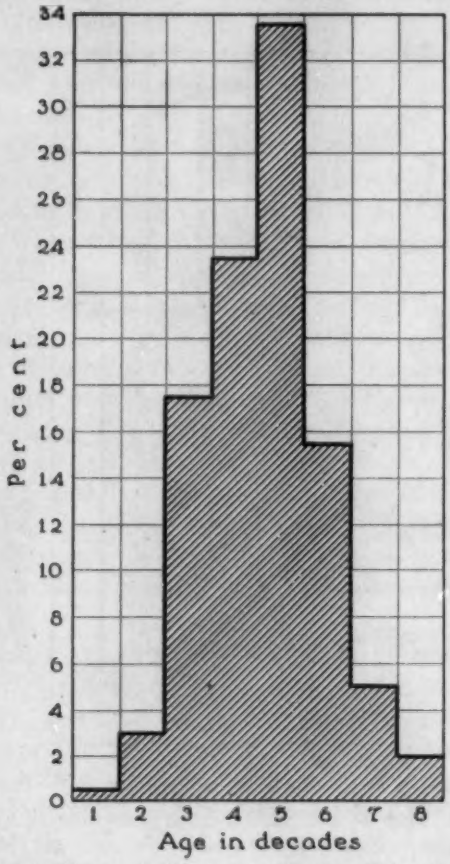


Fig. 2.—Age incidence of old rheumatic valve deformity (160 cases).

longer because the aortic valve is chiefly involved and because the left ventricle can stand an aortic lesion better than a mitral lesion. This question is apparently partly answered by referring to figure 3, which is based on 34 of the 93 persons with the calcified nodular aortic valve deformity. These 34, besides having the aortic lesion, also had a mitral valve deformity of greater or less degree. The greatest number of these 34 died in the fifth decade instead of in the sixth and seventh decades, as did the greatest number of the 93 having the calcified

nodular aortic valves. The percentage of those dying before the fifth decade was 35 instead of 26, and the percentage of those who died in decades above the fourth was 65 as compared with 74. The important thing to be observed in comparing figures 1 and 3 is that with mitral involvement death takes place at an earlier period than when the aortic valve alone is involved. It is also to be noted in observing figure 1 that these calcified nodular aortic valves can be present in relatively young

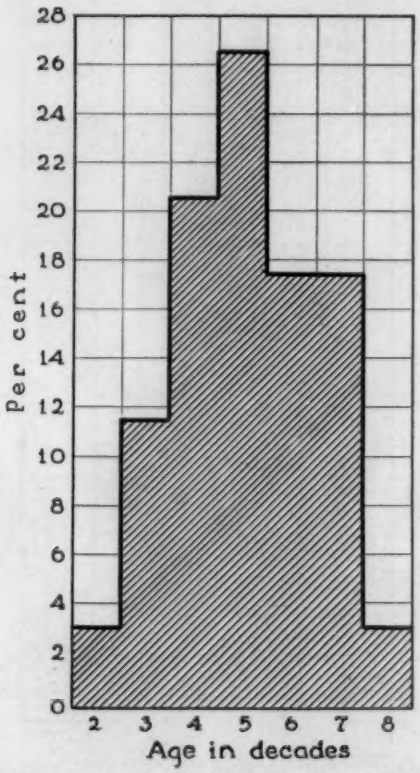


Fig. 3.—Age incidence of calcified nodular aortic deformity associated with mitral lesion (34 cases).

people, 1 per cent in the second decade, 7.5 per cent in the third decade and 18 per cent in the fourth decade, or 47 per cent in the first five decades. It is strongly suggested that the reason old persons so commonly have the aortic calcified type of lesion is because persons having the aortic valve deformity as the chief valvular lesion can live longer, because their hearts can compensate longer, than when the main lesion is on the mitral valve.

The condition of the arch of the aorta strongly suggests that the calcified nodular condition of the aortic valves, in most cases, begins

rather early in life. The smoothness of the intima is a conspicuous finding in at least 90 per cent of the cases. It is apparent that these deformed valves in some way offer a protection to the aorta and lessen the chance of the development of arteriosclerosis. Since the degree of senile arteriosclerosis of the aorta is decidedly less in persons with calcified aortic valve deformities than in persons of a similar age without them, it seems evident that the injury to the aortic cusps begins in most cases early in life. The fact that the calcified nodular aortic type of lesion is common in old persons cannot be used as evidence to indicate that such aortic lesions are due to metabolic changes that occur chiefly in old persons.

An objection sometimes offered to the theory of infection in the etiology of calcified nodular aortic valve deformities is that the evidence of acute rheumatic fever in the group is slight. It is therefore necessary to inquire concerning the frequency of rheumatism in these cases. Three things indicative of previous attacks of acute rheumatic fever are a positive history of rheumatism, evidence of nonspecific pericarditis (acute or healed) at autopsy and the association of rheumatic lesions on one or more of the other valves. Rheumatism, as indicated by a positive history or by evidence of a previous pericarditis, or by both, was present in 49 per cent of the 160 cases of the noncalcified nodular type of old valve deformity and in 40 per cent of the 93 cases of the calcified nodular valve deformity, or nearly as frequently in the latter as in the accepted old rheumatic cases. No group of noncardiac cases coming to autopsy would have as high an incidence of adherent pericardium as is found in this group of cases of calcified nodular aortic valve deformity.

The association of rheumatic lesions, acute or healed, in one or more of the other valves also suggests that the calcified nodular aortic valve deformity, as well as the deformities on mitral, tricuspid or pulmonary valves, may be due to infection with the rheumatic virus. Four of the persons with calcified nodular aortic valves had acute rheumatic vegetations on the mitral valve. There were healed lesions in the mitral valve in 34 of the 93 persons with calcified nodular aortic valves. Three not included in this series of 93 had bacterial vegetations engrafted on the calcified nodular aortic valves.

The frequent evidence of previous attacks of rheumatic fever in the group with the calcified nodular valves would seem to support the infectious rather than the metabolic nature of the valve changes, especially since the incidence of rheumatic fever is practically as great as in the group in which the rheumatic virus is regularly accepted as the etiologic agent.

GROSS STRUCTURES OF THE VALVES

Another approach toward information concerning the etiology of these calcified nodular aortic valve deformities is to study the gross

appearance of the valves. An estimation of the degree of diffuse thickening of the cusps is important. Sixty-eight of the 93 hearts were preserved and were available for careful study. The degree of diffuse thickening in the cusps in these 68 hearts is shown in table 2.

It is to be observed that all but 2 of the 68 hearts showed some degree of diffuse thickening of the cusps. In about 80 per cent there was a severe degree of thickening. Typical arteriosclerotic changes were commonly found in all the valves, especially in the mitral; but thickening of the cusps was not associated with the arteriosclerotic changes. On the other hand, repeated attacks of rheumatic valvulitis definitely bring about thickening. It seems reasonable to assume that the diffuse thickening in the cusps in the calcified nodular type of valves is most probably due also to attacks of rheumatic valvulitis, since the calcified nodular valve deformity is the outstanding type when the chief involvement is in the aortic valve.

TABLE 2.—*Degree of Diffuse Thickening of the Cusps in Calcified Nodular Aortic Valves in Sixty-Eight Cases*

	Cases	
Degree	Number	Per Cent
0	2	3.0
+	12	17.5
++	25	37.0
+++	29	42.5
Total	68	100.0

Grade + indicates a relatively small amount of thickening. Grade +++ is an extreme degree of thickening, and grade ++ is intermediate.

Another gross anatomic consideration of importance in studying the etiology of valve deformities is whether the cusps are fused one to the other. In the 68 hearts examined, fusion of the cusps from a slight to an extreme extent was present in 88 per cent. It was often extensive enough to cause severe stenosis. This fusion suggested an inflammatory rather than a metabolic basis for the changes in the valves.

The location of the calcified nodules in the cusps is considered significant by some observers. Mönckeberg [2] emphasized the fact that in his cases the nodules were on the aortic surface of the cusps. This suggested to him that the process was carried over from the aorta, because the fibrous layer of the cusps on the aortic side was a continuous structure with the aorta. In our cases, the relative frequency of the calcified nodules on the aortic side alone, on the ventricular side of the cusps alone, or on both sides was as shown in table 3.

It is seen that the frequency of nodules on the aortic surface alone was greater than on the ventricular surface alone, but neither condition was common. A greater frequency on the aortic surface would be likely, regardless of the etiologic factor, since the aortic half of the cusp consists of a denser structure and vessels are to be seen in this half

much less frequently. Calcification occurs in tissues poor in blood supply. The frequency of the nodules existing within the cusps and extending outward so as to be evident on both sides is high, 88 per cent. The location of the calcified nodules in the thickened cusps does not seem in any way to support the arteriosclerotic basis for the etiology of the valve deformities as suggested by Mönckeberg.[2] The location of the nodules in the thickened cusps, on the other hand, fits in with an inflammatory process with scar formation that becomes calcified.

The location of the calcified nodules in relation to the angle joining the aorta and the cusps was considered important by Mönckeberg. He believed that the angle was the usual location of the nodules and thought that the nodule was continuous with the arteriosclerotic process in the aorta. Acute rheumatic vegetations are generally found on the ventricular surface of the cusps at about one third the distance from the free margin to the base of the cusps. In the cases of calcified nodular aortic valve deformity that I studied, the nodules were in the angle in 23 per cent. In the remaining 77 per cent of the cases, they were

TABLE 3.—*Location of Calcified Nodules on Aortic Cusps in Sixty-Eight Cases*

Aortic Surface Alone		Ventricular Surface Alone		Both Surfaces	
Number	Per Cent	Number	Per Cent	Number	Per Cent
7	10	1	1.5	60	88

located chiefly in the level that is most frequently attacked by acute rheumatic vegetations. The location of the calcified nodules on the aortic cusps seems to favor an infectious rather than a metabolic process as the etiologic factor in producing the calcified nodular type of valve deformity.

Nothing in the gross structure of the valves seems to indicate an arteriosclerotic or a metabolic basis for the anatomic change. On the other hand, all changes noted are what might be expected in a healed scarred area due to inflammation. The gross appearance of the calcified nodular aortic valves does not seem to bear any relation to arteriosclerosis, but it does bear a definite resemblance to the gross structure of calcified mitral valve deformities, which are generally accepted as being the result of rheumatic infection.

MICROSCOPIC STRUCTURE OF THE VALVES

The calcified nodular valves were studied microscopically, and the structure was compared with that of valves the defects of which were known to be of rheumatic origin. In a previous study, Clawson and Bell [4] found the microscopic appearance in old rheumatic valves to vary from stages in which the polyblastic cellular reaction was almost as active as in acute rheumatic valvulitis, to those in which practically nothing was left but a hyalinized scar. A polyblastic cellular reaction

in which varying numbers of mononuclear and polynucleated polyblasts with various degrees of activity are found is a definite indication of an inflammatory process. The calcified nodular valves in 35 cases were studied microscopically. In 22 (63 per cent) there was a definite polyblastic type of reaction. In 13 (37 per cent) the structure consisted of a scar without evidence of an active inflammation. This relative proportion of active polyblastic reactions to definitely healed scars is practically the same as that found in old rheumatic mitral valves. The polyblastic reaction seems evidently not to be a foreign body reaction, due to the presence of calcium in these valves, for the reaction bears no definite relation to the position of the calcium. The reaction may be pronounced in the absence of calcium or there may be large deposits of calcium without any polyblastic cellular reaction.

One of the most conspicuous things noted in a healed scarred old rheumatic valve is the presence of blood vessels. Blood vessels are not seen in normal aortic cusps by microscopic examination. Vessels easily seen with the microscope develop in old rheumatic scarred valves evidently as a result of inflammation. Thirty-five valves of the calcified nodular type were examined microscopically for the presence of blood vessels. In 32 (91.5 per cent) blood vessels large enough to be seen easily with the low power lens were present. These blood vessels, like the polyblastic cellular reaction, bore no relation to the position of the calcium. The presence of bone was not uncommon. This appeared to be a result of inflammation. Osseous tissue is not infrequently encountered in healed pulmonary tuberculous lesions. Cholesterol crystals, so conspicuous in the intima of arteries in arteriosclerosis, were not found in any of the 35 valves examined.

The microscopic structure of the calcified nodular aortic valves was similar to that of valves with defects known to be due to rheumatic inflammation. There was no resemblance to the structure of arteries with arteriosclerosis. The changes seemed evidently to be due to inflammation of rheumatic origin and not to a metabolic or to an arteriosclerotic process.

COMMENT

Ninety-three cases of calcified nodular aortic valve deformity were compared with 160 cases of noncalcified nodular valve deformity. The purpose of this comparison was to obtain information in respect to the frequency, the etiology and the pathogenesis of the calcified nodular aortic valve deformity (the so-called arteriosclerotic valve).

The calcified nodular type of aortic valve deformity is of common occurrence. In fact, this type is the usual one on the aortic cusps. Stenosis of the aortic ring is frequently found. Clinically a diagnosis of aortic stenosis should be made. If it is not, a high percentage of old valve deformities will not be properly diagnosed.

The time at which death occurs in this group does not seem to be of any significance in respect to the etiology of the process. While it is true that death occurs at a later age in persons having the calcified nodular aortic valve deformity than in those having healed mitral lesions, yet it is to be observed that nearly half of such persons die by the time that they are 50 years old. The reason that this type of valve lesion is more common in old persons than the noncalcified nodular type seems to be explained by the fact that a heart with an aortic lesion will compensate longer than a heart with a mitral deformity, or with an aortic and a mitral deformity, and by the fact that aortic valve deformities of severe involvement are generally of the calcified nodular type.

The gross findings in these valves, such as diffuse thickening and fusion of the cusps, and the location of the calcified nodules in the cusps, all tend to support the inflammatory rather than the metabolic basis for the changes in the valves. Likewise, the microscopic structures, as the polyblastic cellular reaction, the presence of blood vessels within the injured cusps and the absence of cholesterol crystals, strongly support the infectious basis in the etiology of the calcified nodular aortic valve deformities. There does not seem to be any indication for considering the changes in the valves as due to a metabolic process such as arteriosclerosis.

SUMMARY

Aortic valve deformities of severe involvement are usually of the calcified nodular type.

An aortic stenosis of severe grade is a common finding with this type of valve deformity. Clinically, a diagnosis of aortic stenosis should frequently be made.

The reason the calcified nodular type of aortic valvular deformity is so common in older people seems to be that the heart will compensate better with an aortic deformity than with a mitral deformity and most severe aortic valve deformities are of the calcified nodular type.

The frequency of rheumatism in these cases, as indicated by a previous positive history, by the presence of an adherent pericardium or by an association of deformities in other valves, strongly suggests an infectious basis for the calcified nodular valve deformity.

The gross and microscopic structure of these calcified nodular aortic valves indicates that the process is infectious.

There seems to be no support for the metabolic theory of the structural changes in these valves.

It is doubtful whether a valve deformity severe enough to cause cardiac insufficiency is ever due to a metabolic disturbance such as arteriosclerosis.

The term arteriosclerotic valve deformity should not be used in describing valvular insufficiency or stenosis.

HOMOLOGOUS LIVER AS A STIMULUS TO HEPATIC REGENERATION *

FRANK A. McJUNKIN, M.D.
AND
HERBERT C. BREUHAUS, B.S.
CHICAGO

A normal regenerative capacity is often demonstrable in both spontaneous and experimental lesions of various parenchymal organs. That control is exercised by the body as a whole is shown by the constant ratio of body weight to organ weights. The stimulus that initiates the regeneration and the control mechanism that stops it are often imperfectly understood. In neoplastic growth the tumor cells are not under the complete control of the regulatory mechanism that limits proliferation. Once the blastoma has arisen the failure of the host to limit its growth is apparently due to a defect in the tumor cell, since successive hosts behave similarly to the transplanted tumor. The exact character of the defect and its method of origin are not known. Although various factors playing rôles in blastomatous and nonblastomatous growth are known, the ultimate mitotic stimulus and its reactions within the cell are unknown. Our experiments were made to test the effect of the lytic products of a cell on its regeneration.

Much information has been obtained in regard to the stimulant action of various tissue derivatives on cells proliferating in vitro. Carrel and Baker [1] found that the regeneration of skin of the guinea-pig was much accelerated by the addition of proteose and other primary derivatives of protein to the tissue culture mediums. To these investigators especially is due the knowledge that polypeptides stimulate cell growth. The addition of other substances, such as vitamins, to cultures of sarcoma tissue caused no acceleration of growth. Carrel [2] stated that growth-promoting substances of the general nature of polypeptides are present in the various organs, and that they may be manufactured by leukocytes from fibrin and cell débris in inflammatory processes and wounds. Hammet [3] has extensively investigated the stimulant effect of compounds containing the sulphydryl

* Submitted for publication, July 20, 1931.

* From the Department of Pathology, Loyola University School of Medicine.

1. Carrel, A., and Baker, L. E.: J. Exper. Med. **44**:503, 1926. Baker, L. E., and Carrel, A.: ibid. **47**:353, 1928.

2. Carrel, A.: Proc. Inst. Med., Chicago **8**:62, 1930.

3. Hammet, F. S.: Protoplasm **7**:20, 1929.

group, and is of the opinion that this chemical group alone is active in the promotion of growth. Voegtlin and Chalkley [4] added glutathione, which is a tripeptide containing the sulphydryl group to a nonnutrient medium of Amoeba proteus and observed an increase in the rate of proliferation. Such findings suggest that the stimulus for cell regeneration is of a chemical nature. Gurwitsch [5] recently advanced the theory that cell division is stimulated by ultraviolet rays given off by living cells.

Within the body, the cells are evidently in an environment more complex than in the tissue culture. The plasma in which the body cells are bathed may contain not only growth-promoting substances, but also inhibitory agents active in the regulation of cell growth. In fact Carrel [2] recognized an inhibitory effect of plasma that increased with the age of the animal. In spite of this complex situation, the composition of the plasma may be varied by the enteric or parenteric administration of various substances. This Loeb [6] performed in the case of the thyroid gland by feeding guinea-pigs subjected to partial thyroidectomy with thyroid gland. The result was that the regenerative activity of the thyroid remnant was inhibited. Potassium iodide, on the other hand, not only failed to inhibit compensatory hypertrophy, but actually stimulated it.[7] Although small amounts of protein may pass through the intestinal wall unchanged, most of it is subjected to extensive lytic changes, and only the less complex compounds, such as thyroxine, reach the blood stream. In our experiments, parenteric administration was employed in order to eliminate the digestive action of the gastrointestinal tract. The experiments may be divided into two general groups, the first consisting of partial hepatectomies and ligations and the second of intraperitoneal injections of crushed liver into animals otherwise normal. The white rats used in all of the experiments were obtained originally from the Wistar Institute, and during the past several years had been inbred to a considerable extent. All animals were kept on the same adequate uniform diet.

REGENERATION OF HEPATIC CELLS AFTER HEPATECTOMY AND AFTER LIGATION OF THE LIVER

The operative procedure for both the partial removals and the ligations was simple. A 5 cm. length of linen tape, 4 mm. wide, was folded, and the middle portion pushed up beneath the diaphragm and the uppermost of the three left lobes. One of the free ends of the ligature was then carried toward the left, encircling the lobes to be

4. Voegtlin, C., and Chalkley, H. W.: Pub. Health Rep. **45**:3041, 1930.
5. Gurwitsch, A.: Arch. f. Entwcklngsmechn. d. Organ. **100**:11, 1923.
6. Loeb, L.: J. M. Research **41**:481, 1920.
7. Loeb, L.: J. M. Research **40**:199, 1919.

ligated, and the other end was pressed into the large fissure separating these lobes from the remainder of the liver. The ligature was then drawn tight and tied. In this way, the circulation to the three left lobes was shut off. In the hepatectomies, the ligated lobes were cut off close to the ligature. The total mortality at first reached 20 per cent, but later was decreased somewhat with some variation according to subsequent procedures. Usually when the animals were alive and doing well on the third day, they continued so. The restoration of the liver to its normal weight after partial hepatectomy is quite well understood. Higgins and Anderson [8] and Higgins and Priestley [9] found that the liver was restored to its normal weight in about three weeks after the removal of from 65 to 75 per cent. These investigators determined the actual weight of the regenerating liver at stated periods after the hepatectomy. The weight is influenced by the blood content and the amount of other cellular elements, especially of reticulo-endothelium and erythroblasts. Since we were especially interested in the hepatic cells, their mitotic activity was determined directly. At the termination of the experiment the entire liver was fixed in 10 per cent formaldehyde, and blocks were cut at right angles to the large surfaces so as to include both the thick diaphragmatic portion and the thin margins. With the mechanical stage, all mitoses in two or three sections were enumerated, and the average per section was set down in tables.

Not only were mitoses absent in animals that died from the experimental procedures, but they were usually absent in animals killed while obviously sick. In the dog, autolytic liver has been found to be highly toxic, with fatal results following the intraperitoneal injection of 100 Gm. Andrews and Hrdina [10] made a careful study of the fatal peritonitis produced by injections of liver, and reviewed the work of the earlier investigators of the lesion. The peritonitis consisted of an outpouring of sanguineous fluid containing little pus and little fibrin, which was associated with an overwhelming gas bacillus infection. They interpreted the fatal peritonitis as the result of some toxic agent contained in both autolyzed and fresh liver that created a great increase in the permeability of the bowel to Bacillus welchii and a rapid infection of the liver. They found the toxic factor to be water-soluble and thermostabile and regarded it as a proteose or a peptone. Apparently the rat is much less susceptible to invasion by Bacillus welchii, but in many of our dead animals an appearance in the peritoneal cavity identical

8. Higgins, G. M., and Anderson, R. M.: Proc. Staff Meet., Mayo Clin. **5**:243, 1930.

9. Higgins, G. M., and Priestley, J. T.: Proc. Staff Meet., Mayo Clin. **6**:249, 1931.

10. Andrews, E., and Hrdina, Leo: Surg., Gynec. & Obst. **52**:61, 1931.

with that described in the dog was found. Had we found a decrease in regeneration in the animals that received liver, the results would not have been easy to interpret, on account of the possible toxic effect of the peritonitis.

The weight of the three left lobes represented from 30 to 45 per cent of the total weight of the liver. On the first and second days after the removal of this amount of liver few mitoses were found. After this time, regeneration developed and continued moderately active for two weeks (table 1). With the exception of rat 128, none of the rats presented numerous mitoses. The large number of mitoses in rat 128 was not explained. For the eleven animals in this group the average number of mitoses was 5.3 per section, as compared with an average of 0.04 for thirty-two normal rats (table 2). In our normal rats relatively few mitoses were present. In rats weighing under 150 Gm. (table 2,

TABLE 1.—*Cell Mitoses in Livers of Rats Subjected to Hepatectomy*

Rat	Weight, Gm.	Days Following Hepatectomy	Mitoses*
104	120	7	1.0
106	125	3	0.0
113	150	4	0.0
115	230	5	0.0
118	200	9	0.5
125	145	3	1.5
127	180	6	1.0
128	210	9	45.5
129	265	9	8.0
130	365	2	0.5
131	215	9	0.5

* Average number in sections.

nos. 142, 143, 148, 147, 149, 150, 187, 191, 192, 193, 194 and 195), 1 or 2 mitoses were found; the largest number was 5. In a majority of the normal rats no mitoses were present in the two or three sections examined. The experiments show that the removal of about one third of the liver is followed, after the first three or four days, by a mitotic activity greater than that seen in normal, young, actively growing rats. Early in the work the effect of local trauma on the liver was studied by snipping small bits from the edge and surface of the liver (first twelve rats in table 2). In rat 24, 1 or 2 mitoses per section were found, but these were not in the vicinity of the wound. The hepatic cells manifested no tendency to regenerate, when injured locally, and in this respect they differed from the epithelia of the skin and mucous membrane surfaces.

The ligations were identical with the partial hepatectomies, except that the ligated lobes were not removed. A small amount of blood passed through the ligature, since certain structures, including some of the bile ducts, remained alive in the periportal tissue. However, prac-

tically all of the ligated liver was necrotic at the end of twenty-four hours and at the end of two days showed distinct evidence of solution. In from three to five days, the trabeculae of the hepatic cells were largely dissolved. Since there were no gross breaks on the surfaces of the ligated lobes, it seemed likely that the dissolved substances passed into the general circulation through the blood vessels not completely closed by the ligature. In this way, the dissolved liver entered the general circulation and was carried to the intact portions. In the thirteen rats

TABLE 2.—*Cell Mitoses in Livers of Rats Subjected to Local Snipping of Hepatic Tissue and in Livers of Normal Rats*

Rat	Weight, Gm.	Days Following Local Injury to Liver	Mitoses*	Comment
19	198	2	0.0	Snip
21	186	1	0.0	Snip
24	134	3	1.0	Snip
29	100	1	0.0	Snip
37	94	1	0.0	Snip
40	96	1	0.0	Snip
61	166	2	0.0	Snip
62	262	3	0.0	Snip
63	106	3	0.0	Snip
65	210	4	0.0	Snip
66	240	2	0.0	Snip
68	240	4	0.0	Snip
142	94	..	0.0	Normal
143	118	..	0.0	Normal
148	150	..	2.0	Normal
147	140	..	2.0	Normal
149	100	..	5.0	Normal
150	110	..	0.5	Normal
181	220	..	0.5	Normal
182	200	..	0.0	Normal
184	190	..	0.0	Normal
185	200	..	0.0	Normal
186	180	..	0.0	Normal
187	150	..	0.0	Normal
188	160	..	0.0	Normal
189	160	..	0.0	Normal
190	100	..	0.0	Normal
191	99	..	0.0	Normal
192	114	..	1.0	Normal
193	112	..	0.0	Normal
194	108	..	0.5	Normal
195	105	..	0.0	Normal

*Average number in sections.

(table 3) the average number of mitoses per section was 11.1 as compared with 5.3 in the rats with hepatectomies. Perhaps the most noticeable difference seen in a comparison of tables 1 and 3 is the regular presence of 3 or more mitoses from the third to the fourteenth day in the rats with ligations.

EFFECT OF INJECTIONS OF HOMOLOGOUS LIVER ON REGENERATION OF HEPATIC CELLS AFTER HEPATECTOMY

The method of preparing the liver for injection through a large, short needle with a lumen 1.5 mm. in diameter was to remove the entire liver aseptically and crush it with a pestle in a sterile mortar. Some-

times the addition of a few drops of distilled water was necessary to bring the macerated mass to the proper consistency for injection. The hepatectomy was performed by the method described. On the second day, the macerated liver was injected intraperitoneally into all the rats (except rat 169), and into four of the rats a second dose was injected. The results, shown in table 4, were the most decisive obtained. With the exception of a heavy older rat (no. 169), all the rats showed the hepatic

TABLE 3.—*Cell Mitoses in Ligated Livers of Rats*

Rat	Weight, Gm.	Days Following Ligation	Mitoses*
108	140	3	7.0
110	212	5	4.0
111	225	20	0.0
112	170	14	3.0
120	190	4	7.0
121	190	1	0.0
122	210	6	38.0
123	190	7	26.0
124	190	7	3.0
135	200	8	5.0
138	215	9	27.0
141	203	6	21.5
144	224	28	3.0

* Average number in sections.

TABLE 4.—*Cell Mitoses in Livers of Rats Subjected to Hepatectomy and Treated by Injection of Homologous Liver*

Rat	Weight, Gm.	Days Following Hepatectomy	Amount Macerated Liver Injected, Gm.	Mitoses*
155	210	5	7.0 (2nd day)	7.0
156	225	5	7.0 (2nd day)	38.0
157	235	7	7.0 (2nd day)	5.5
158	150	7	7.0 (2nd day)	64.0
160	150	8	12.5 (2nd and 6th days)	205.0
162	182	9	6.5 (6th day)	344.0
163	190	9	12.5 (2nd and 6th days)	156.0
169	380	11	15.5 (5th and 7th days)	0.0
172	220	12	15.5 (2nd and 8th days)	297.0

* Average number in sections.

cells actively proliferating, and in the nine rats the average number of mitoses was 124 per section. In none of the rats with simple hepatectomies or ligations did the number reach 100. In single fields seen under the high power dry lens, more mitoses were usually present than in entire sections of the livers of rats not treated by injection of liver, from which the same percentage of liver had been removed (table 1). In some fields almost one-half the hepatic cells were in karyorrhexis. So far as could be determined from the relatively small number of animals in the series, the hepatectomized rats withstood the injections approximately as well as the nonhepatectomized normal rats of the same weights.

EFFECT OF INJECTIONS OF HOMOLOGOUS LIVER ON HEPATIC CELLS IN NORMAL RATS

An attempt was made to induce proliferation in the hepatic cells of normal rats by injections of liver. When 6 Gm. or more of crushed liver was injected as a single dose into the peritoneal cavities of rats weighing from 150 to 200 Gm., a considerable mortality resulted from the serosanguineous peritonitis. In general, the normal rat tolerated the large doses scarcely as well as the hepatectomized animals. In rats weighing about 200 Gm. that were given an injection of 6 Gm. of liver, mitoses usually were not found (table 5). In five rats weighing under 175 Gm. each of which had received two doses of 6 Gm. or more, mitoses were found. In normal, untreated rats of this weight, mitoses were usually not found in the routine examination of two sections.

TABLE 5.—*Cell Mitoses in Livers of Normal Rats Treated by Injection of Liver*

Rat	Weight, Gm.	Duration of Observation, Days	Amount Macerated Liver Injected, Gm.	Mitoses*
151	132	7	12.0 (1st and 4th days)	3.5
152	150	8	12.0 (1st and 4th days)	8.3
154	164	7	14.0 (1st and 4th days)	23.3
174	195	9	15.0 (1st and 5th days)	5.0
176	170	7	13.5 (1st and 4th days)	1.0
177	200	4	6.0 (1st day)	0.0
179	200	7	6.0 (1st day)	....
180	325	4	7.5 (1st day)	0.0

* Average number in sections.

COMMENT

The view is prevalent that necrotic tissue even in the absence of bacterial invasion is toxic. The local leukocytic reaction about necrotic tissue and certain systemic reactions, such as elevation of temperature following infarction, are cited as toxic manifestations. The data obtained by tissue culture methods cast doubt on this conception. Carrel and Baker [11] showed conclusively that the products of proteolysis, such as the polypeptides, are growth-promoting, although the individual amino-acids do not stimulate growth. In the body, where conditions are less simple, few tissue products have been tested to determine their effect on specific types of living cells. Particulate matter, as shown by the intraperitoneal injection of suspensoids and also coarser suspensions, such as that of red blood corpuscles, quickly passes from the peritoneal cavity into the lymphatics and may reach the blood stream. Foreign protein, of course, readily enters the blood from the peritoneal cavity. If one may judge from the autolytic changes observed microscopically in the injected liver, the protoplasm of injected hepatic cells reaches the

11. Carrel and Baker (footnote 1, first reference).

liver of the host in solution by way of the general circulation. McJunkin and Matsui,[12] investigating the local action of dead macerated epidermis on the regeneration of epidermis, found that it stimulated this regeneration, and that the stimulant effect was somewhat specific, since it did not follow the application of macerated liver.

It seems to us that the chief environmental difference between cells proliferating in vitro and those proliferating in vivo is the contact of the latter with a constantly changing plasma. This makes possible a continuous local removal by the cells of a stimulating or an inhibiting substance supplied to the circulating blood elsewhere. In our experiments, the dissolved protoplasm of a particular type of cell plus any disintegration products formed from such cells was added to the plasma, and within the liver it stimulated mitosis. The substance presumably acts as a chemical stimulus or as a physicochemical excitant. The current explanation offered for regeneration of parenchymal tissue is that of physiologic need or functional necessity. It is not apparent that the macerated tissues increase the functional requirements. It is more plausible to view the formative stimulus as a food element. Relative differences between types of cells are apparent. In the skin there is an immediate proliferative response to correct any loss of epidermal cells. Carrel [2] held that substances such as those present in the embryo and among the decomposition products of fibrin and dead tissue are directly produced by the injury and by the subsequent action of enzymes, and that these substances stimulate cells to regenerate. Unlike the epidermal cells the hepatic cells do not regenerate in response to local injury (twelve rats in table 2). This difference in regenerative activity may be inherent in the cells, but there is an obvious difference in environment. Granulation tissue forms in the hepatic wound; but its vascularity does not equal the efficient normal sinusoidal blood supply. In cutaneous wounds, the granulation tissue delivers an excess of blood as compared with the normal dermis. Here again a pabulum theory may be employed to explain the results, although it offers no ultimate explanation for cell division. In cellular metabolism specific cell products are constructed from the general food supply. Based on our experiments, certain of these products may be recognized as specific food substances. The plasma may contain these specific products, perhaps only in traces, as well as the general nutritive substances. Higgins and Priestley [9] emphasized the importance of increased blood in the regeneration following partial hepatectomy. An increase in blood supply would deliver to the epidermoid or to the hepatic cells not only additional general nutrition, but also added amounts of any "type-cell food" present in the

12. McJunkin, F. A., and Matsui, T.: Effect of Homologous Macerated Skin on the Regeneration of Epidermis, Arch. Path. **12**:794, 1931.

plasma. With a relative increase of the latter in the plasma, the proliferation of cells might be stimulated without an increase in the blood supply. The greatly increased rate of regeneration in the hepatectomized animals treated by injection of liver over that in animals with simple hepatectomies is best explained by a qualitative rather than a quantitative change in the blood, since injections of macerated liver do not appear to increase the flow of blood through the liver. In the destructive lesions of parenchymal organs often associated with regeneration, products from the necrotic parenchyma enter the circulation. If it is assumed that the various "type-cell foods" are present in traces in the circulation, a decrease in the total amount of such tissue by extirpation would make available a relatively greater amount of this specific nutriment for the cells in the remnant of the gland. The formative stimuli appear not to be of the nature of glandular secretions or hormones. Indeed, the experiments of Loeb [6] with thyroid feeding suggest that such products may be inhibitory. Finally, it must be kept in mind that present knowledge does not warrant the assumption that cell regeneration and its control or lack of control rests entirely on the supply of nutritive elements, general and specific, or on the exhaustion of this supply.

CONCLUSIONS

In the rat subjected to partial hepatectomy, injections of homologous macerated liver greatly increase the regeneration of hepatic cells.

Absorption of the rat's own ligated, necrotic liver leads to some increase of karyorrhexis in the remnant.

Maximum parenteral injections of homologous liver into half-grown rats stimulate somewhat the proliferative activity of the liver.

The dissolved protoplasm of cells (hepatic) contains a type-cell formative stimulant.

ASBESTOSIS

REPORT OF TWO CASES *

HAROLD L. STEWART, M.D.

CARL J. BUCHER, M.D.
AND
ERNEST H. COLEMAN, M.D.
PHILADELPHIA

Asbestos as such was known to Pliny. Charlemagne is said to have possessed a table cloth made of it, which was cleansed by passing through fire. The mineral deposits of asbestos are found in Canada, Italy, Africa and Rhodesia. It is a silicate occurring in minerals in combination with iron, copper, calcium or magnesium. It occurs in the form of fibers which can be woven. In addition, finer particles are used in the manufacture of sheeting, tiles, asbestos millboard and paper. The increasing need for brake linings for motor cars, fireproof curtains, insulations, mattresses and steam packings has expanded the spinning industry considerably. The use of the dust in cement and plaster for the erection of fireproof buildings has increased the danger of inhalation in the most hazardous form of the industry. Its expansion from 500 tons in 1880 to 330,000 tons in 1925 and the fact that methods of manufacture have been introduced for utilizing the fine dust for new purposes have contributed to the beginning appearance of a pneumokoniosis due to inhalation of asbestos dust,[1] known as pulmonary asbestosis.

In 1906, Montague Murray [2] presented evidence before the Departmental Committee on Compensation for Industrial Diseases, that he had had under his care in 1900 a worker employed in an asbestos factory who developed respiratory symptoms and died. At the autopsy, extensive pulmonary fibrosis was found, which Murray attributed to inhalation of asbestos dust.

In 1924, eighteen years later, Cooke [3] reported a similar case complicated by pulmonary tuberculosis. He was the first to describe the

* Submitted for publication, July 17, 1931.

* From the Department of Pathology, Jefferson Medical College and Hospital, aided by a grant from the Martin Research Fund.

1. Merewether, E. R. A.: J. Indust. Hyg. **12**:198, 1930.

2. Murray, Montague: Departmental Committee on Compensation for Industrial Diseases, Minutes of Evidence, Appendices and Index, 1907, Cd. 3496, p. 127; Report 1907, Cd. 3495, p. 14.

3. Cooke, W. E.: Brit. M. J. **2**:147, 1924; **2**:1024, 1927.

"curious bodies" in the lung now known as "asbestosis bodies," while McDonald [4] worked out the histologic features. The latter believed that the bodies were portions of asbestos in process of alteration and absorption by hydrolysis, with the passing of silica into a colloidal state and later a gel. Stewart and Stewart and Haddow [5] showed that these bodies could be found in the juices expressed from affected lungs and could be found in the sputum by digesting it with equal quantities of antiformin. Lynch and Smith,[6] working without knowledge of this, demonstrated these bodies by digesting sputum with 10 per cent sodium hydroxide. Gloyne [7] proved by means of dark-ground illumination, that when the golden-yellow asbestosis body was dissolved in concentrated sulphuric acid, it had a central core consisting of a minute asbestos fiber. The asbestos fiber itself under dark-ground illumination has the appearance of a sharp piece of wire. Fibers measuring 200 microns [8] can pass the protective mechanism of the upper respiratory tract and enter the lung.

REPORT OF CASES

Case 1.—J. M., a white man, entered the Jefferson Medical College Hospital on June 8, 1931, in the service of Dr. Edward Klopp. He complained of headache, abdominal distention and eructation of acid and gas. A diagnosis of chronic cholecystitis was made. A cholecystectomy was performed. Peritonitis developed, and the patient died. The history disclosed certain facts relative to asbestosis. The patient had worked as a spinner in an asbestos mill for nine years. Although the atmosphere was not very dusty, he wore silver dust protectors in his nostrils. For five years following such employment, he worked as a laborer. During the past five years, he had morning cough with moderate expectoration. There was no history of hemoptysis or pain in the chest. He had some dyspnea and cardiac palpitation. Physical examination was unimportant with the exception that he had an emphysematous type of chest. The important roentgen observations were slightly increased peribronchial markings throughout both lungs and some calcific deposits in both root areas. The cardiac shadow appeared to be normal. The sputum was not examined.

At the postmortem examination, the heart appeared normal. The left lung weighed 270 Gm. and the right 320 Gm. They were bluish gray, practically free from adhesions and crepitant throughout. On section, no gross pathologic lesion was demonstrable. Microscopically, there was a moderate grade of emphysema.

---

4. McDonald, S.: Brit. M. J. **2**:1025, 1927.

5. Stewart, M. J.: Brit. M. J. **2**:509, 1928; **2**:581, 1929. Stewart, M. J., and Haddow, A. C.: J. Path. & Bact. **31**:172, 1929.

6. Lynch, K. M., and Smith, W. A.: J. A. M. A. **95**:659, 1930.

7. Gloyne, S. R.: Tubercle **10**:404, 1929.

8. Wood, W. B., and Gloyne, S. R.: Lancet **1**:445, 1930.

Some of the alveolar walls were slightly thickened. A few typical asbestosis bodies were seen in the alveolar walls. There were scattered small collections of brown and blackish dust, free and within phagocytic cells, in fibrosed areas. Much of this dust and all of the asbestosis bodies stained positively for iron. The liver and the spleen were not pigmented.

The question of the relation of pulmonary asbestosis to tuberculosis is an interesting one. The fact that they are frequently associated is settled. Whether tuberculosis becomes implanted on pulmonary asbestosis or whether the occupational disease lights up a quiescent lesion is a mooted question.[1] It is true that when tuberculosis occurs, it is liable to be of an anomalous type. Gardner and Cummings [9] exposed series of guinea-pigs to attenuated tubercle bacilli and to an atmosphere laden with asbestos dust and attenuated tubercle bacilli. They found, in the first instance, that normal guinea-pigs receiving the strain of attenuated tubercle bacilli showed tubercles in the lung and in the tracheobronchial lymph nodes comparable to the primary complex in man. The lesions caseated and healed by resolution. Spread of the infection with macroscopic disease in other viscera was rare. On exposing guinea-pigs to attenuated tubercle bacilli and to asbestos dust, 32.2 per cent of one group of animals showed some evidence of spreading tuberculosis. New disease began as a local extension from primary tubercles and metastasized to areas where dust reaction had occurred. The tendency to healing by fibrosis was marked. Macroscopic disease in the spleen was common and occurred occasionally in the liver. In another group receiving asbestos and attenuated tubercle bacilli, the localization of tubercles was atypical. Many bacilli were seen trapped in foci of dust reaction. Some produced local tubercles; others immediately entered dilated lymph vessels and were carried to the tracheobronchial lymph nodes. Tuberculosis of these nodes sometimes occurred without involvement of the lung. Early disease of the spleen and of hepatic lymph nodes occurred in the majority of cases. The combined action of tubercle bacilli and asbestosis in the lung produced more fibrosis than did either agent acting independently.

Case 2.—J. J., aged 52, entered a hospital in December, 1928. In 1921, he worked in an asbestos factory for a period of about nine months. In this factory, no precautions were taken against inhalation of asbestos dust. Exactly what his duties were was impossible to determine. On admission, he complained of fatigue, loss of weight and a slightly productive cough. Tubercle bacilli were demonstrable in his sputum at that time. On roentgen examination, a cavity in the right upper portion of the chest and a fine stippling in the lower portions of the lower lung

9. Gardner, L. W., and Cummings, D. E.: J. Indust. Hyg. **13**:65, 1931.

fields suggesting pneumokoniosis were found. There was improvement; the patient was discharged and continued under observation as an ambulatory patient. In September, 1930, he was admitted to the Jefferson Hospital for Diseases of the Chest, in the service of Dr. B. Gordon. He complained of the same symptoms as before. Physical examinations revealed the following salient facts: He was markedly emaciated. The chest was emphysematous in type. Expansion was much limited, particularly in the right upper portion, where there was lagging. Over the apex of the right lung, physical signs indicative of a cavity were present. Over the remainder of both lungs, expansion was limited; tactile fremitus was increased; breath sounds were roughened and somewhat exaggerated, and there were numerous scattered râles. The area of cardiac dulness was within normal limits; the heart sounds were distant; there was a mitral murmur at the apex. The abdomen appeared to be normal. There were clubbing of the fingers and curving of the nails; the skin was slightly cyanotic. The patient left the hospital in March, 1931, against medical advice, somewhat improved, but he returned a month later with exacerbation of all his symptoms and an acute respiratory infection. His course was progressively downward; bronchopneumonia developed, and he died, April 2, 1931. At no time during his stay in Jefferson Hospital were tubercle bacilli found in his sputum. There were a moderate secondary anemia, a slight elevation of nonprotein nitrogen, and negative results from Kahn and Wassermann tests. On roentgen examination, Sept. 20, 1931, there was multiple small cavitation in the right upper lobe, and the interlobar pleura below it was much thickened. In the lower lobe, right, and in the middle and lower lobes, left, there was an increase in the markings that resembled a dust change. The heart appeared normal.

At the postmortem examination, the heart weighed 280 Gm. and measured 13 by 8 by 6 cm. The myocardium was mottled red and yellow, and appeared soft and dull. There were many firm, gray, translucent nodules on the free margins of the mitral valve leaflets. The coronaries were thickened and tortuous, and contained atheromatous plaques. The parietal pleura on both sides was thickly studded with small, discrete, gray, pinhead-sized nodules, firm in consistency. Similar ones were present, but much less frequently, on the visceral pleura. Over the bases of both lungs, firm fibrous adhesions completely obliterated the pleural cavities. Between the bases of the lungs and the diaphragm, these adhesions were converted into a thick, tough, yellow, homogeneous substance having the appearance of hyaline cartilage. The right lung weighed 960 Gm. and the left 830 Gm. The consistency of both lungs was much increased over the normal. The bases were particularly firm and tough, and dark brown. The upper half of the right upper lobe was excavated by a large multilocular cavity. The wall of this did not differ in density from that of the adjacent lung tissue, giving the impression that the cavity had formed so rapidly that a limiting fibrous wall was not thrown out. The cavity measured 6 cm. across, and through it coursed many thrombosed blood vessels. It had a foul, fetid odor as of gangrene. Throughout the remainder of this lung there were patchy areas elevated slightly above the cut surface, gray and red, moist, and measuring about 1 cm. in diameter. In the left lung, in addition to what has already been described for it, were two round nodules, similar in appearance, one in the upper portion of the lower lobe and one in the lower portion of the upper lobe on the outer aspect near the pleura. The one in the lower lobe was slightly larger, measuring 4 cm. in diameter. It cut with increased resistance and on section was composed of thick, dense strands of fibrous tissue, intermingled with gray, cheesy material. The regional lymph node draining this area was similar to this nodule. There were no cavities in this lung. In many fresh smears examined from these caseous areas, no tubercle bacilli were found.

Microscopically, the parietal pleura was studded with tubercles. These were composed of epithelioid cells, a few lymphocytes, giant cells and fibrous tissue in considerable amounts. Many were hyalinized to a considerable extent. Caseation necrosis was practically absent. They were of the productive type, with a few well formed tubercles.

The nodules in the left lung were large, caseous areas, divided up into lobules by strands of fibrous tissue. Sections from them and from the tubercles in the pleura were examined for tubercle bacilli, but none were found. In sections taken from the large cavity in the upper lobe of the right lung there were fresh lique-

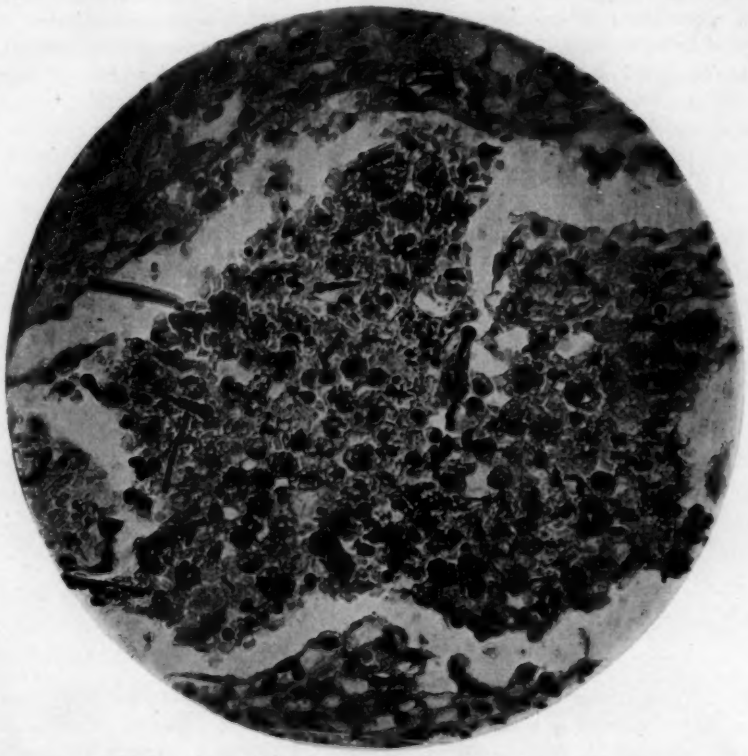


Fig. 1 (case 2).—A small bronchiole completely filled with cellular exudate and "asbestosis bodies." The fine segmentation and the clubbed end in some can be distinctly seen; × 400.

faction necrosis, very little polymorphonuclear and lymphocytic cell infiltration, little hyperemia and a thin, newly formed layer composed chiefly of young fibroblasts.

The principal lesion of the lungs was a diffuse fibrosis, particularly marked in the lower lobes. In many places, the alveolar walls were markedly thickened; in others, the alveoli had been completely replaced by fibrous tissue. Many bronchi were inflamed, and there were many areas of pneumonia present. A high percentage of the arteries were the seat of an obliterative endarteritis. The most striking feature in the lungs was the "asbestosis bodies." They were present in great numbers in the fibrosed areas, in some alveolar walls and spaces and in the

smaller bronchial branches. Particles of them were fragmented and were seen in phagocytic cells. Mainly, they lay loose in small clusters with collections of polymorphonuclear leukocytes and polyblasts about them, often completely occluding a small bronchiole or an alveolar duct. They measured roughly from 10 to 200 microns and were golden brown. They had a variety of appearances, but a characteristic morphology. They were seen often as a series of regular disks very much as are red blood cells in rouleaux formation. Frequently a series of disks ended in a club form on one or on both ends. Sometimes they were seen as long delicate filaments ending in clubbed ends; or again as short clubbed forms, single or double, suggesting a dumbbell. Occasionally, single, paired or coccal forms in chains were found, or even sporelike aggregations. They were present in the juice squeezed from the lungs. They did not stain with hematoxylin-eosin or Gram's stain. They did, however, give the prussian blue reaction for iron in varying degrees of intensity, in the main rather pronounced. In the caseous tuberculous areas in the left lung, these asbestosis bodies were fewer in number than in the adjacent lung tissue. A few were normal, but many were pale with indistinct

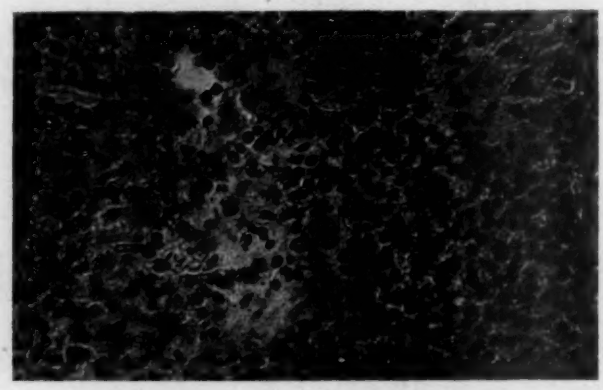


Fig. 2 (case 2).—Spleen. An asbestosis body is lying in the center of the field. One end is clubbed; the other pointed; × 400.

outline. When these caseous areas had been stained for pressian blue reaction following the staining of them for acid-fast bacteria (carbol fuchsin saturated solution 95 per cent alcohol, decolorization with 25 per cent nitric acid, Loeffler's methylene blue), the asbestosis bodies stained a pale green instead of a distinct blue, and their morphology was less regular, but they could still be recognized. In addition to the asbestosis bodies there was considerable golden-brown and black, granular pigment present in phagocytic cells. Much of this gave the iron reaction.

In the spleen, the reticulo-endothelial cells were loaded with a fine, amorphous golden-brown and sometimes blackish dust. Some of this dust was extracellular. A few large, typical asbestosis bodies were found. Practically every hepatic cell contained a few granules of a golden-yellow pigment. Many of the Kupffer cells contained considerable quantities of light golden-yellow and blackish granules of pigment. About the portal radicles it was common to see a few cells loaded with pigment. In the mucosa of the stomach there were many phagocytic cells filled with fine, golden-brown pigment granules. In the kidney, many cells were seen in the capillaries of the glomeruli which were filled with coarse granules of a golden-brown and blackish pigment. The lymph node draining the caseous area in

the left lung contained a caseous tuberculous area similar to the one described in that lung, and in addition, many well healed tubercles. All bronchial nodes were deeply pigmented and fibrotic. The pigment was in the form of fine brown and black granules, mainly within phagocytic cells, and a great deal of it stained for iron. A few asbestosis bodies were present.

COMMENT

The two cases cited illustrate nicely the extremes and some of the features of the disease. In the first case, as far as the patient's health and life were concerned, the presence of pulmonary asbestosis in the lung was relatively unimportant. He had symptoms doubtfully attributable to it. It was only through the history, which was confirmed by postmortem examination, that the possibility of such a condition was suggested. It is, however, important to point out two considerations in this case. First, it is a question whether the asbestosis had anything to do with his emphysema and cough. As an etiologic factor, the asbestosis cannot be ignored. The other point is that one has to do with preventive medicine. The exposure was for nine years in a comparatively well ventilated factory in which precautionary appliances were used, and yet the patient developed a minor grade of the disease. It would seem that further precautionary means are necessary in this occupation to obviate a disease in itself so preventable.

In the second case, the patient worked in an asbestos factory for a short time, but without a mask. This was long enough to permit his lungs to become densely filled with the dust. Obviously the symptoms did not result from the asbestos per se, but were due to the marked fibrosis resulting from the deposition of the pigment. This demonstrates that the symptoms of a pneumokoniosis may come on years after a short exposure and emphasizes again the absolute necessity for wearing masks in even the less dusty atmospheres.

The marked fibrosis of the pleura, particularly of that interposed between the base of the lung and the diaphragm, is characteristic. The finding of rapidly liquefying areas in the lung is not unusual either, but the presence of such an extensive freshly excavated area is.

We have been able to demonstrate two large tuberculous lesions in the midlung just beneath the pleura, tuberculosis of the regional lymph nodes and tuberculosis of the parietal pleura. The analogy between this form of lesion and that of the tuberculosis of childhood is, to say the least, striking. Gardner and Cummings have shown the course of asbestos inhalation with coincident tuberculous inoculations. Dr. Gardner, in a personal communication to us, replied in the negative to our question as to whether or not any experimental work had been done on the influence of asbestos inhalation on a healed primary focus. It seems to us this would throw light on the subject, for it does not appear that the asbestosis and the tuberculosis were coincidental lesions.

Our second case confirms previous observations that the asbestosis bodies have a tendency to disappear in a caseous area. We have also shown that in such an area following an acid-fast stain (25 per cent nitric acid) the bodies do not stain so well for iron and have a less definite morphology, but are still recognizable as such, so that if these are present in the sputum in any case, they should be recognized on a routine smear even after it has been treated with acid.

The finding of tubercle bacilli in the sputum in the second case on two different occasions, plus the gross and microscopic features characteristic of tuberculosis, is sufficient to establish such a diagnosis. It is significant, however, that many more specimens of sputums were examined for acid-fast bacilli in which none were found, and that no one noted the asbestosis bodies in the sputum, although these were not being looked for specifically.

An important additional finding in case 2 was that of asbestosis bodies in the spleen. Although we have diligently searched the literature, mention of such a finding, to our knowledge, never previously has been made. Whether these bodies get there by the blood stream as emboli or whether they are carried there by phagocytic cells, we are not prepared to say and have no evidence on which to base a conclusion. The fact that they are in that organ, irrespective of how they got there, seems to be significant.

It is interesting that neither in case 1 nor in case 2 (despite the marked pulmonary fibrosis and the thickening of the smaller pulmonary arteries) was there any dilatation or hypertrophy of the heart on either side.

SUMMARY

Two cases of pulmonary asbestosis are reported. In the first case, the symptoms were relatively unimportant. In the second case, the disease was concomitant with tuberculosis and, with it, was the cause of death. The similarity between some of the features of tuberculosis in childhood and tuberculosis complicated by asbestosis is suggested.

In asbestosis the lung is not the only organ invaded by asbestosis bodies. As we have shown, the spleen and the lymph nodes may harbor large asbestosis bodies.

From the histories there appears to be a further need for study along the lines of prevention of this disease.

TULAREMIC LEPTOMENINGITIS

REPORT OF A CASE *

ARTHUR R. BRYANT, M.D.
The John Jay Borland Fellow in Medicine
AND
EDWIN F. HIRSCH, M.D.
CHICAGO

Francis,[1] in 1928, tabulated twenty-four fatal tularemic infections in man and stated that five of the patients died in the second week of illness in delirium and stupor, the symptoms indicating severe involvement of the meninges (meningitis). The reports in those cases in which necropsies were made say nothing about the brain. Francis and Callender,[2] in their report, had in mind the possibility of lesions of the meninges or of the brain in their reference to the postmortem examination of a Negro in whom tissue reactions resembling those of tularemia were found in the spleen. Because of stupor and other clinical symptoms of meningitis, death was ascribed to tuberculosis. The meninges at the base of the brain were opaque, and microscopically there was a diffuse infiltration by lymphocytes, with considerable thickening and an occasional Langhans' giant cell surrounded by a few epithelioid cells. There was a hyperplasia of the endothelial cells of the capillaries. Francis and Callender stated that the lesion may have been tuberculous, although acid-fast bacilli were not demonstrated. Examinations of the blood and inoculations of guinea-pigs were not made. In the brain of a woman dying on the eighth day after the onset of illness, Palmer and Hansmann[3] noted hyperemia, a minute hemorrhage of the pons, small masses of endothelial cells in the pia-arachnoid, fatty changes of the neurons, glia cells and muscle cells of the blood vessels and a diffuse degeneration of the myelin in the fiber tracts of the pons and in the fibers of the eighth cranial nerve. Replying to an inquiry by Dr. Warren T. Vaughan on involvement of the central nervous system in tularemia, Simpson[4] stated that he did not think that there is an actual

* Submitted for publication, July 27, 1931.
* From the Henry Baird Favill Laboratory of St. Luke's Hospital.

1. Francis, E.: J. A. M. A. **91**:1155, 1928.
2. Francis, E., and Callender, G. R.: Arch. Path. **3**:577, 1927.
3. Palmer, H. D., and Hansmann, G. H.: J. A. M. A. **91**:236, 1928.
4. Simpson, W. M.: J. Lab. & Clin. Med. **15**:311, 1930.

involvement of the central nervous system, but that the nervousness, restlessness and insomnia of the disease are associated with the general debility. Dwijkoff [5] observed regularly a marked hyperemia of the meninges and brain in rodents with tularemia, and sometimes small hemorrhages. In the meninges of one animal, he found a large hemorrhage and a small infiltration.

It is reasonable to expect in a septicemic disease such as tularemia that almost every tissue of the body at some stage of the illness may be infected. While these general references have been made to clinical symptoms of meningitis in tularemia, there seems to be, as yet, no report of reactions of the meninges or of the brain comparable in cell structure to the lesions regularly found in the liver, spleen and lungs of susceptible hosts infected with Bacterium tularense. A description of such lesions distributed in the meninges, submeningeal brain tissues, ependyma, subependymal tissues and choroid plexus of a man who died on the sixteenth day after infection is recorded in the following paragraphs.

REPORT OF CASE

W. S., white, aged 48, a chef, entered St. Luke's Hospital, Chicago, in the service of Dr. F. M. Miller, Dec. 14, 1930, with a rapid pulse, a high fever, an ulcerated wound of the right middle finger, tenderness of the right axilla and 7,100 leukocytes per cubic millimeter of blood. On December 5, while he was removing pickled rabbit from a jar, the skin on the dorsal surface of the right middle finger opposite the first interphalangeal joint was torn on a bone of the rabbit. The lesion was immediately treated with a solution of iodine. The following day he was indisposed, and two days later he was acutely ill. The wound of the finger was opened, and hot fomentations were applied, but these had little effect on the chills and fever and on a painful swelling in the right axilla. The chills and fever persisted while he was in the hospital, and on the second day after his admission symptoms of meningitis developed. The patient became irrational. The neck was rigid; the pupils were small and sluggish; the Kernig, left Babinski and Oppenheim reflexes were present; the cremasteric reflex was absent, and the knee reflexes were diminished. The spinal fluid was cloudy and under a pressure of 230 mm. of water, it contained 400 cells per cubic millimeter of fluid, of which 16 per cent were polymorphonuclear leukocytes and 84 per cent lymphocytes. The results of globulin tests were positive, and the albumin content was 75 mg. per hundred cubic centimeters. Bacteria were not found in the sediment, stained by Gram's and Ziehl-Neelsen's stains. Cultures of the spinal fluid on the usual mediums were sterile after six days.

Death occurred on December 21, the sixteenth day after infection of the finger, and the postmortem examination of the viscera of the trunk and head, but not of the neck, was made the same day by Dr. Paul J. Breslich and Dr. J. J. Kearns. The essentials of the anatomic diagnosis were: miliary tularemic lesions of the liver, spleen and lungs; marked acute ulcerative and phlegmonous cellulitis of the right middle finger; acute hyperplasia and necrosis of the right axillary, right supraclavicular and biliary lymph nodes; acute tularemic meningitis; edema of the brain;

5. Dwijkoff, P. P.: Virchows Arch. f. path. Anat. **278**:481, 1930.

marked cloudy swelling of the liver, kidneys and myocardium; marked fatty changes of the liver and kidneys; acute hyperplasia of the spleen; acute fibrinous perisplenitis; acute catarrhal tracheitis and bronchitis; bilateral bronchopneumonia; bilateral hydrothorax, and no yellow lipoid substance in the suprarenal cortex.

Only the more important observations recorded at autopsy will be mentioned. The body weighed 147 pounds (66.7 Kg.) and was 173 cm. long. There was a slight icterus of the sclerae and of the skin. On the ulnar and dorsal side of the first interphalangeal joint of the right middle finger was an ulcer 15 mm. in diameter. The tissues of the finger were edematous, and the skin was wrinkled from wet dressings. The subcutaneous tissues of the front of the chest were edematous; the abdomen contained 85 Gm. of a clear, yellow fluid. The lymph nodes of the right axilla were as large as 30 by 15 by 10 mm., and on cut surfaces were dark red and about 50 per cent mottled with necrotic regions, some liquefied. The spleen weighed 400 Gm. Many yellow and gray regions, from 1 to 2 mm. in diameter, and petechial hemorrhages were visible through the capsule. The capsule of the diaphragmatic surface was roughened by organizing fibrin. On cut surfaces, the spleen was soft, dark red and mottled with many discrete yellowish-white regions, 1 mm. in diameter. The liver weighed 1,950 Gm. The capsule was smooth. The lobular markings were faint, and disseminated in the brown tissues without regular order were regions varying in color from grayish white to yellow, and measuring 1 mm. or less in diameter. There was marked cloudy swelling of the liver. The biliary lymph nodes were as large as 25 by 15 by 7 mm., and opaque, white lymphoid tissue was mottled with yellow regions of necrosis. The right lung weighed 850 Gm. The pleura was smooth, except dorsally where there were a few torn fibrous adhesions. The lobular margins were pigmented with carbon, and the parenchyma contained many shotty nodules of white tissue from 2 to 5 mm. in diameter. These, on cut surfaces, stood out from the surrounding dark-red lung substance as firm, white, granular nodules resembling miliary tubercles. In addition, there were red, granular bronchopneumonic consolidations of the lung about 1 cm. in diameter. The lining of the bronchi was hyperemic. The left lung weighed 800 Gm. and in all essentials was like the right lung. It had many of the widely disseminated nodules of white tissue. There were no active tuberculous lesions or other significant changes in the viscera except those of tularemia, a conclusion supported by the routine histologic examinations of these tissues, which showed focal tularemic lesions in the lungs, liver, spleen and lymph nodes.

The histologic structure of the lesions in tularemia has been described.[6] The lesions in the liver and spleen of this patient were in the stage in which necrosis and nuclear fragmentation of the exudate cells are predominant. The lesions of the lungs had, in addition, reactive changes along their margins.

Serum removed from the patient on the fourteenth day of the illness, two days before death, was sent to the Hygienic Laboratory of the United States Public Health Service and was reported to agglutinate Bacterium tularense in dilutions not greater than 1:40. Dr. Francis, in a comment on this report, stated that "while the titre is low, it is in keeping with that of serum collected on the fourteenth day of tularemia, and especially in a person who has failed to form antibodies of any sort as indicated by his death on the sixteenth day of illness."

---

6. Francis and Callender (footnote 2). Goodpasture, E. W., and House, J.: Am. J. Path. **4**:213, 1928. Simpson, W. M.: Arch. Path. **6**:553, 1928.

Two guinea-pigs (1 and 2) were inoculated on December 22 with crushed spleen. Both animals died on December 27, and the spleen, liver and lung had focal lesions characteristic of tularemia. Guinea-pig 3, inoculated with splenic pulp of guinea pig 1, died on Jan. 19, 1931, and at the site of the inoculation was granulation tissue microscopically identical with that found in the lesions of guinea-pig 1. Guinea-pig 4 was inoculated on January 27 with crushed spleen obtained from the body of the patient and stored in the icebox for one month. This animal remained healthy. B. tularense was not isolated in cultures of the human and animal tissues. Human lung, liver and spleen and also liver and spleen from guinea-pig 1 were sent to Dr. Francis for examination, who stated that the guinea-pig tissues had the usual lesions found with tularemia. The human liver and spleen had similar focal lesions, and the lungs an exudative pneumonitis and in one place a focal tularemic lesion.

These conditions in the viscera of the trunk are given with considerable detail in order to present adequately the evidence favoring the tularemic nature of the disease. They are important, of course, but are incidental to the chief portions of this report, namely, those that follow, describing the lesions of the meninges and brain.

There were no changes in the blood sinuses of the dura or in the right and left middle ears or in the sphenoid, ethmoid and frontal cranial sinuses. A large amount of slightly turbid spinal fluid escaped when the brain was removed. The hyperemic leptomeninges were slightly opaque with a diffuse exudate and contained many miliary opacities from 1 to 2 mm. in diameter and less. After hardening in 8 per cent formaldehyde, the brain weighed 1,270 Gm. The hemispheres were asymmetric: the right was 17 cm. long, 8 cm. wide and 7 cm. thick in the temporal region; the left, 17 cm. long, 8.5 cm. wide and 6.5 cm. thick. The convolutions were flattened, the sulci shallow and narrow. The brain substance was uniformly firm. There was a milky opacity of the leptomeninges covering the superior and lateral surfaces of the hemispheres, especially along the sulci and in the fissures of Sylvius. Focally, they were raised slightly from the underlying cortex and contained discrete white nodules from 1 to 2 mm. in diameter, especially along the course of the blood vessels in the precentral and postcentral gyri. The blood vessels of the leptomeninges were distended with blood. The basilar and vertebral arteries were absent. The circle of Willis was intact and unchanged. The basal leptomeninges had a milky opacity and were raised from the convolutions and thickened. The olfactory bulbs, optic chiasma and third, fourth, fifth and sixth cranial nerves, the anterior and posterior perforating substance with their blood vessels, the infundibulum and the mammillary bodies were unchanged. The cerebellar peduncles were symmetrical, measuring 2.2 by 1.7 cm. The red nuclei and the substantia nigra were unchanged. The pineal body was 8 by 5 by 5.5 mm., the tissue firm and granulated.

With a binocular magnification of 10.5 diameters, the leptomeninges had disseminated milky opacities, especially distinct over the blood vessels in the sulci; over the blood vessels, they were edematous and lifted away from the cortex. Also along the blood vessels and adherent to or forming a part of the meninges were small multiform plaques, 1 mm. in diameter, like tubercles, but less opaque, and milky white. The opacity of these plaques was so great that the underlying blood vessels were hidden or had a yellow tinge. The opacity of the meninges and the opaque plaques extended over the base of the brain, but not to such a marked extent.

The cerebrum was sectioned frontally in parallel planes about 1 cm. apart. There was a slight increase of the stippling. The gray cortex had an average thickness of from 2.5 to 5 mm. and was sharply demarcated from the white substance. There were no hemorrhages or soft regions in the tissues of the basal ganglions. The lateral ventricles were symmetrical and small, and at the level of the anterior commissure they were 2.8 cm. wide. The third ventricle was unchanged. The ependymal lining of the third and especially of the lateral ventricle was roughened by slightly raised, white nodules less than 1 mm. in diameter. The choroid plexus varied in color from gray to brown and was unchanged. The cerebellar hemispheres were symmetrical and measured 5 by 6 by 5 cm. The pons was 3 by 3.5 by 3 cm. and had 3.8 cm. of the brain stem attached. The pons, brain stem and cerebellum showed no noteworthy changes.

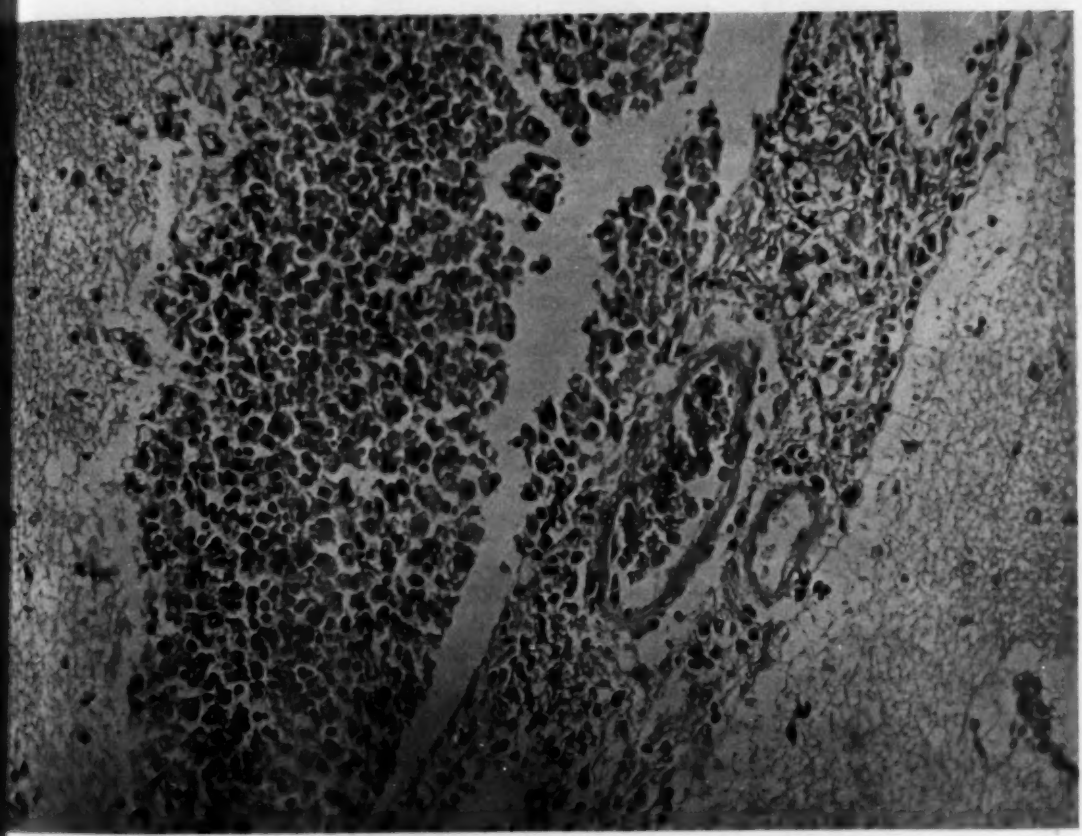


Fig. 1.—A focal lesion of the meninges deep in a sulcus, with involvement of the brain; × 252.

Twenty-eight blocks of tissue were taken for histologic examination from various places of the cortex, the white substance, the principle ganglion centers and tracts, the cerebellum and the choroid plexus of the lateral and third ventricles. They were embedded in paraffin, and most of the sections were stained with hema-

toxylin and eosin, a few with phosphotungstic acid-hematoxylin and otherwise. The nodular opacities in the leptomeninges were masses of exudate cells, chiefly polymorphonuclear leukocytes, large mononuclear cells, plasma cells and cells with a large, faintly stained nucleus and a granular, fairly abundant cytoplasm (fig. 1). These cells were in dense aggregates from 0.5 to 1 mm. in diameter, and were held in meshes of fibrin threads. Most of these focal lesions were in the leptomeninges, but some encroached on the adjacent brain substance and penetrated to a depth

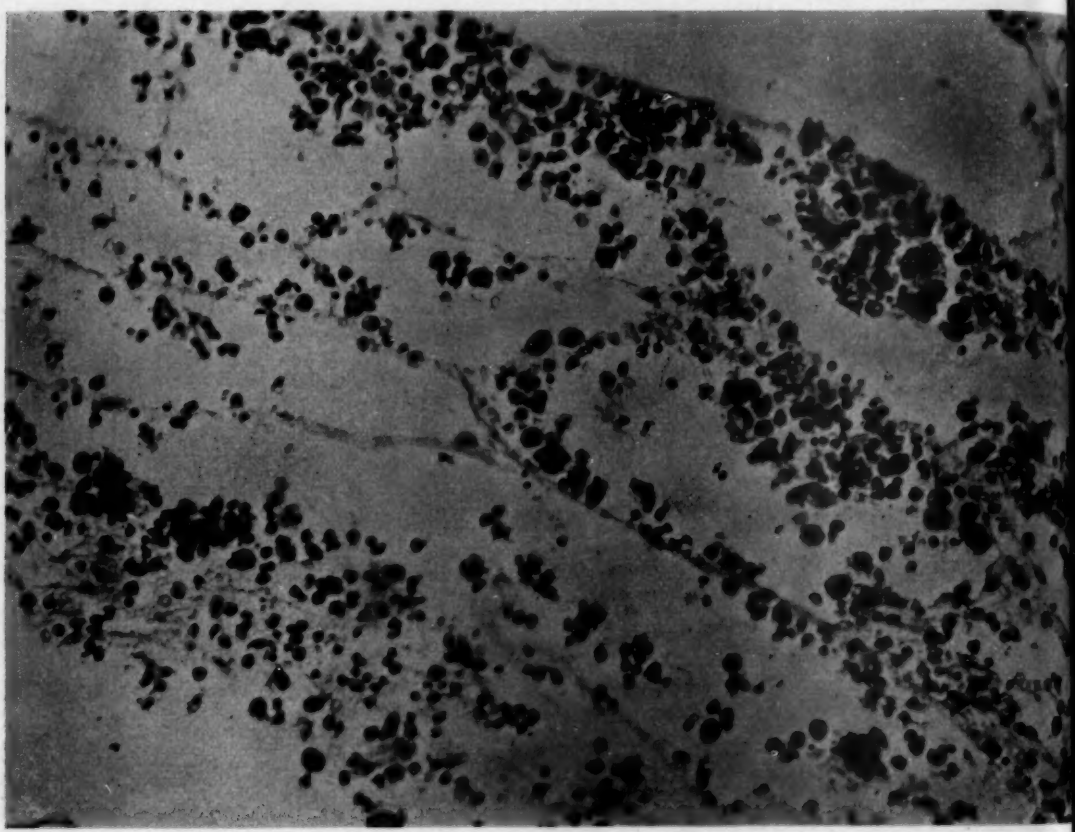


Fig. 2.—A diffuse exudative reaction in the meninges; × 252.

of from 0.2 to 0.5 mm. In such places the brain tissues were necrotic and infiltrated by dense masses of polymorphonuclear leukocytes and large mononuclear exudate cells. The nuclei of many of the exudate cells were pyknotic and fragmented. A study of all these sections demonstrated that the lesions were distributed chiefly, although not invariably, along the blood vessels. In addition to these focal lesions there were diffuse exudative cellular reactions of certain portions of the leptomeninges (fig. 2). Focal lesions were present also on the ependymal lining of the lateral and third ventricle and in the epithelium of the choroid plexus. They were not found isolated in the white substance of the cerebrum or in that of the cerebellum. The leptomeninges of the spinal cord of the first segment had lesions like

those described in the pia-arachnoid of the brain. Acid-fast bacilli were not found in lesions of the liver, spleen and leptomeninges (from three separate places) stained according to a technic that in control tuberculous tissue demonstrated acid-fast organisms.

SUMMARY

Tularemic lesions of the leptomeninges, contiguous brain tissues, ependyma, subependymal tissues and choroid plexus of a man dying on the sixteenth day following laceration of a finger by contact with a rabbit bone had the histologic structure of similar lesions in the liver, spleen and lungs, and corresponded structurally with those described in the visceral tissues of susceptible hosts dying with B. tularense infection.

The lesions grossly resembled miliary tubercles, although their distribution was chiefly over the vertex and sides rather than along the base of the brain, and consisted of exudates of monocytes and polymorphonuclear leukocytes with varying degrees of necrosis. Where the meningeal lesions encroached on the brain, the tissues of the latter were necrotic and infiltrated by exudate cells.

In addition to the focal lesions of the meninges there was a diffuse, acute exudative meningitis. Widely disseminated lesions of the brain were not demonstrated.

Although, generally speaking, the lesions of tularemia mimic those of tuberculosis, it is unlikely that the reactions found in the meninges of this patient were tuberculous, because (1) they repeated in minute detail the structure of lesions found in the spleen, and suspensions of these crushed tissue killed guinea-pigs in five days and in them produced visceral lesions characteristic of tularemia; (2) the search for acid-fast bacilli in the lesions of the meninges, liver and spleen was not successful, and (3) lesions of a frankly tuberculous character were not found by thorough gross and histologic examinations of the viscera.[7]

7. Since the completion of this manuscript, Dr. F. W. Hartman of the Henry Ford Hospital, Detroit, has sent us a copy of his report concerning tularemic encephalitis. His forthcoming article describes lesions in the brain substance, but none in the meninges. In this respect, his observations differ from those in our report.

EXPERIMENTAL ARTERITIS AND ARTERIOSCLEROSIS ASSOCIATED WITH STREPTOCOCCAL INOCULATIONS*

ROBERT L. BENSON, M.D.

KENNETH G. SMITH, M.D.

AND

HERMAN SEMENOV, M.D.

PORTLAND, ORE.

In 1920, one of us observed early aortic arteriosclerosis in a young girl, 14 years of age, with gangrenous stomatitis and fatal septicemia. Cultures from the mouth before death and of the blood at autopsy yielded a mixture of Streptococcus hemolyticus and Staphylococcus aureus. Autopsy revealed septic infarcts of the heart and lungs, disseminated acute myocarditis, thrombi in the right auricle and ventricle and fresh, patchy fatty degeneration of the aorta and larger arteries, with beginning arteriosclerosis. Microscopic examination disclosed intimal thickening, consisting of atheromatous ulcers and arteriosclerosis, involving the entire circumference of the aorta. The media, and in some places the intima, contained blue-staining streaks of homogenous material, which separated the smooth muscle layers. Sudan III-hematoxylin stains demonstrated a lipoid infiltration of the intima. The typical histologic picture of beginning arteriosclerosis was present, and the question then arose whether it was due to the associated infection.

Some time later, we began to culture the coronary arteries of arteriosclerotic patients, particularly in fatal cases of coronary thrombosis. In three instances, Streptococcus viridans was found in the coronary arteries, although cultures of the blood were negative. From one heart we cultured a sclerotic coronary artery, and from two others thrombi in sclerotic coronary vessels. Subsequent intravenous inoculations of rabbits with the recovered streptococci demonstrated the ability of the latter to persist in the blood stream for from three to five days. We considered that these organisms were probably pathogenic and decided to inoculate a series of rabbits in order to determine their effect on the heart and blood vessels.

---

* Submitted for publication, Aug. 3, 1931.

* From the Department of Pathology, University of Oregon Medical School.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

* Work begun in 1924 under the Noble Wiley Jones Fellowship in Pathology of the University of Oregon Medical School.

INOCULATION OF RABBITS

Series 1.—Litter 1, of seven rabbits, each of which was inoculated intravenously with a massive dose of streptococci from one of the cases, was observed for about a week and then killed. Although blood cultures showed that the organisms could be recovered for from three to five days after inoculation, autopsy disclosed no significant cardiovascular changes, except a slight lymphoid infiltration of the interstitial tissue of the myocardium. As a single massive inoculation failed to produce changes, we decided to make repeated inoculations over a prolonged period of time.

Series 2.—Litter 2 consisted of seven young rabbits. Five received seven inoculations each with Streptococcus fecalis from a coronary artery in a period of five and one-half months. Rabbit 5, in addition to its inoculations, had thirty-three

Fig. 1.—Arteriosclerosis of the aorta of rabbit 5, series 2, inoculated with Streptococcus viridans from a sclerotic coronary artery.

cholesterol feedings of 0.5 Gm. each. Rabbit 6 had only cholesterol, thirty-three feedings. Rabbit 7 was saved as a control. All seven were killed at the end of six months.

Only rabbit 5 showed any gross changes in the aorta (fig. 1). The ascending aorta contained thickened, yellowish, ulcer-like plaques, in which the intima was considerably raised. There was also a more diffuse irregular thickening of the whole ascending aorta and to a lesser extent of the descending aorta. Microscopic examination disclosed decided thickening and irregularity of the intima with considerable fibrous increase and scarring and with obscuring of the lamina elastica interna (fig. 2). Fibroblasts were scattered throughout the intima, and a few vacuoles were present. The intima also contained elongated streaks of blue-staining material. Sudan III-hematoxylin stains disclosed a diffuse infiltration by small globules of lipin throughout the intima. The arteriosclerotic process,

which involved the intima and to some extent the media, had nearly passed the atheromatous phase and had undergone fibrosis with considerable scarring.

Litter 3, consisting of two young rabbits, without controls, received three inoculations with the same strain of S. fecalis, in a period of two months. At autopsy, one of these showed small atherosclerotic plaques in the ascending aorta; the other did not.

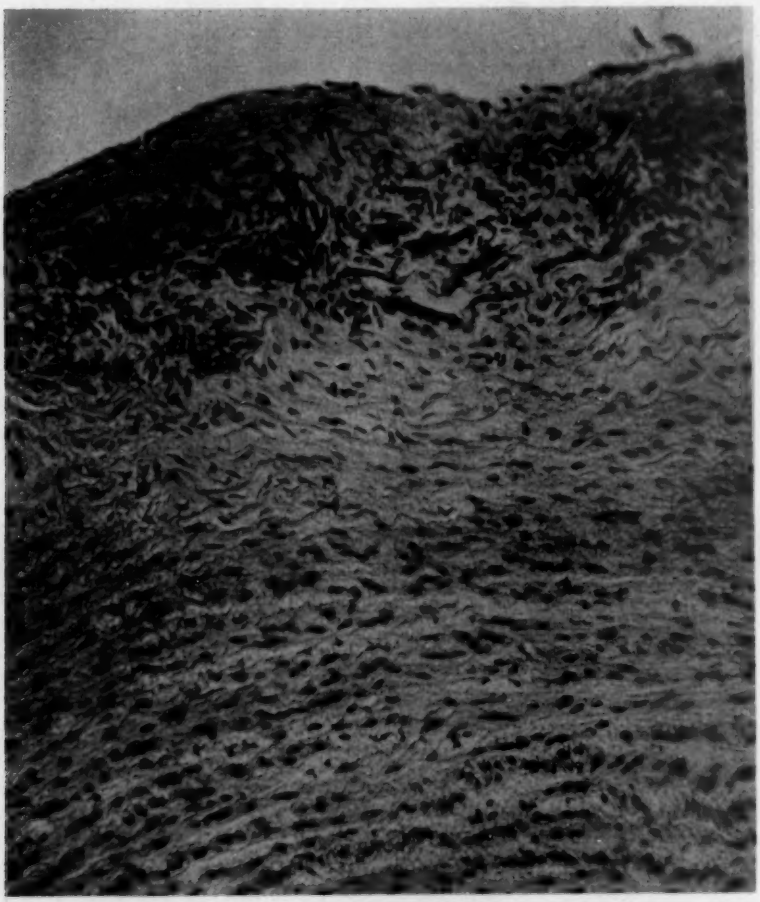


Fig. 2.—Photomicrograph of the aorta of rabbit 5, series 2, showing fibrous thickening and scarring of the intima and cellular infiltration of the adventitia and media.

Litter 4 consisted of six rabbits, two of which were kept as controls. Of the others, three received two inoculations each with the streptococcus, and another received four inoculations. The inoculated animals were also given a subcutaneous implantation of 2 cm. of aseptic rabbit aorta, in the hope of inducing absorption of enough aortic substance to influence the electivity of the injected organisms for aortic tissue. The organisms used had been repeatedly cultivated over a period of many days in dextrose infusion broth containing a portion of sterile

rabbit aorta. One rabbit died one month after inoculation, with atherosclerotic plaques in the aorta. The other five rabbits, including the controls, lived from one to three months and showed no gross lesions.

Litter 5 consisted of seven rabbits. Two were kept as controls, and the others received two inoculations with the streptococcus. Autopsies on all of them, four months later, showed no gross changes.

Series 3, Inoculated with Streptococcus Viridans from a Coronary Thrombus.—A thrombus in the coronary artery of a man, 66 years of age, who died of generalized arteriosclerosis with coronary thrombosis was cultured two hours after the patient's death. At the same time, the blood in the heart was cultured and found to be sterile. The thrombus was cultured in dextrose infusion broth enriched with sterile human blood, and on blood agar plates. The plates yielded many colonies of a green-producing nonhemolytic streptococcus, and the broth gave a good growth of long chain, lanceolate diplostreptococci. This was identified by growth in lactose, mannite and salicin as nonhemolytic streptococcus type III (Holman).

TABLE 1.—*Observations in Litter 6, Inoculated with Strain of Streptococci from Coronary Thrombus*

Rabbit	Procedure	Duration, Mo.	Fate	Aortic Lesions	Lesions of Smaller Arteries	Pathologic Changes
1	27 inoculations with streptococci	8	Died	0	0	Pericarditis
2	27 inoculations with streptococci	10	Killed	0	+	Sclerosis of arteries of lung
3	27 inoculations with streptococci; 127 cholesterol feedings	9	Killed	+	+	Large, flat, whitish atheromatous plaques, microscopically arteriosclerotic
4	27 inoculations with streptococci; 127 cholesterol feedings	8	Killed	0	+	Intimal thickening and sclerosis of small arteries in lung and liver; endarteritis obliterans
5	131 cholesterol feedings.....	9	Killed	0	0	None
6	Control.....	9	Died	0	0	Pneumonia

All rabbits used in our experiments were young, healthy animals, weighing from 2 to 3 pounds (907 to 1,360 Gm.) each, and great care was given to their feeding and hygiene. The diet consisted of fresh vegetables with a regular ration of oatmeal, and whenever cholesterol was fed, it was with oatmeal. On several occasions, inoculations were discontinued for a period of time and repeated blood cultures made to determine the duration of survival of the organisms in each animal's blood stream. This period was found to vary from one to two weeks. In the course of repeated inoculations in the aural vein, abscesses were eventually produced in the tissue of the ear, and these may have accounted for the persistence of the organisms in the blood stream.

Litter 6 consisted of six rabbits. Rabbits 1 and 2 were each inoculated intravenously twenty-seven times in a period of eight months with the streptococcus grown in dextrose infusion broth. Rabbits 3 and 4 were similarly inoculated but, in addition, each received 127 cholesterol feedings of 0.5 Gm. Rabbit 5 had the cholesterol feedings only, and no. 6 was saved as a control. The findings among the various rabbits in this litter are indicated in table 1.

Rabbit 3, which survived nine months of inoculations and cholesterol feedings, presented large, flat, whitish atheromatous plaques of the aorta (fig. 3). Micro-

scopic examination of the aorta showed a thickened intima with lipoid infiltration (fig. 4). Very little cellular infiltration was present. The smaller arteries of the lung, kidney and brain showed irregular thickening with moderate fibrous increase.

Rabbit 4 showed no gross lesions. Microscopic examination disclosed irregular thickening of the walls of the smaller arteries of the lung, with fibrous increase and, in some cases, almost complete closure of the lumen. The proliferation was chiefly intimal, with considerable hyaline change. In some of the arterioles, proliferation of the intimal cells nearly filled the lumen, and in other cases the occlusion was completed by a hyaline substance. In the larger arteries, cellular proliferation was evident and a crescentic thickening of the intima sometimes encroached appreciably on the lumen, almost occluding it. In the lung, three arteries, one about 2 mm., and the others 1 mm., in diameter, were infiltrated by

Fig. 3.—Extensive early arteriosclerosis in the aorta of rabbit 3, litter 6, inoculated with Streptococcus viridans from a coronary thrombus.

mononuclears and polymorphonuclears. This was especially true of the intima, which was thickened, hyalinized and densely infiltrated by polymorphonuclears, and which blended off into a laminated red thrombus that occluded the dilated lumen of the artery (fig. 5). Portions of the thrombus contained dense accumulations of pyknotic nuclei. In the kidney, there was thickening of the intima of the arteries with endothelial cell proliferation and lipoid infiltration. The walls were infiltrated by clusters of lymphoid cells and polymorphonuclears. The arterial findings included endarteritis obliterans, arteriosclerosis, mainly intimal, and endarteritis of branches of the pulmonary artery, with occluding thrombosis. Rabbit 2 also had sclerosis of the smaller arteries of the lung.

Litter 7 consisted of six rabbits. Rabbits 1 and 2 were inoculated twenty-seven times each in eight months with the streptococcus. Rabbits 3 and 4, in

addition to these inoculations received cholesterol feedings of 0.5 Gm. each. Rabbit 5 had only cholesterol feedings, and rabbit 6 was saved as a control (table 2).

Rabbit 3 survived ten months and had small, whitish sclerotic plaques in the ascending aorta. In rabbit 4, one arterial branch in the lung was nearly occluded by an old thrombus with fibrosis. A large area of lung in the immediate vicinity of this vessel was necrotic. Several other arteries had thickened walls with intimal proliferation (fig. 6).

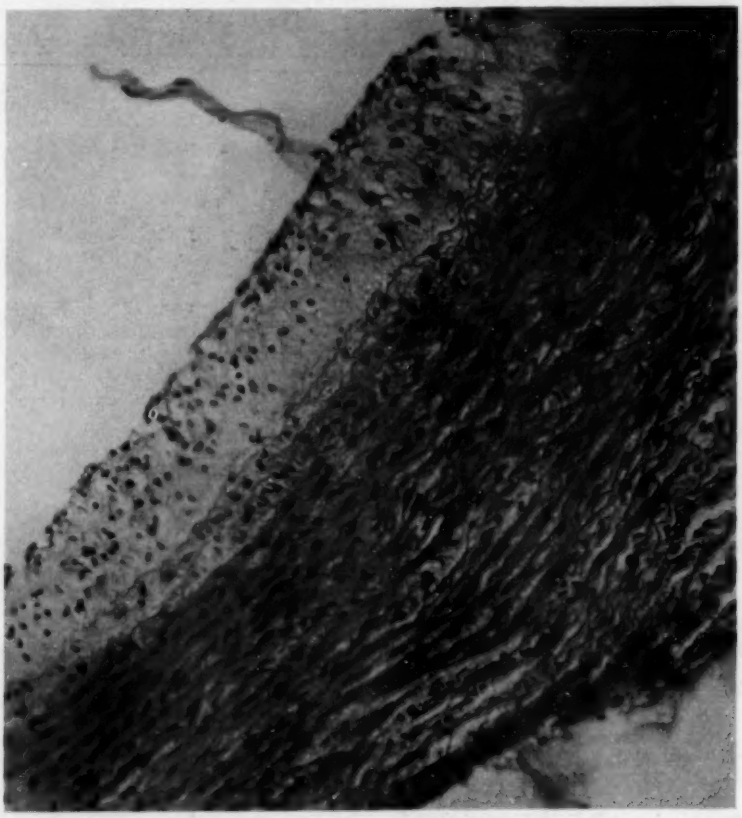


Fig. 4.—Photomicrograph of the aorta of rabbit 3, litter 6, showing moderately early arteriosclerosis with thickening of the intima characterized by loose tissue.

Litter 8 consisted of five rabbits. Each of these was inoculated intravenously seven times with the streptococcus over a period of about seven months (table 3).

Rabbit 2 showed indurated ulcers of the aorta. Microscopic study revealed early arteriosclerosis of the aorta. One small artery in the lung had a thick wall, with roughening of the intima on one side and an attached thrombus of fibrin and polymorphonuclears filling one third of the lumen. Rabbit 1 exhibited slight intimal thickening of the smaller arteries of the lung. Rabbit 3 showed intimal thickening and hyaline change in a medium-sized branch of a coronary artery, with dense, patchy lymphoid infiltration of the media. The arterial findings

revealed chronic indurative arteritis and endarteritis with hyaline changes and irregular scarring of the walls. The control rabbit, no. 5, died of pneumonia after two months' observation, and microscopic examination disclosed thickening of the intima and media in the smaller arteries of the lung, a slight thickening of a branch of a coronary artery and acute glomerulotubular nephritis. It was possible that the arterial changes and nephritis in this rabbit resulted from the pneumonia.

TABLE 2.—*Observations in Litter 7, Inoculated with Strain of Streptococci From Coronary Thrombus*

Rabbit	Procedure	Duration, Mo.	Fate	Aortic Lesions	Lesions of Smaller Arteries	Pathologic Changes
1	27 inoculations with streptococci	8	Died	0	0	Bronchopneumonia
2	27 inoculations with streptococci	10	Killed	0	0	None
3	27 inoculations with streptococci; 131 cholesterol feedings	10	Killed	+	0	Small sclerotic plaques of aorta
4	23 inoculations with streptococci; 62 cholesterol feedings	3	Died	0	+	Arteriosclerosis and thrombosis of medium-sized arteries of lung; infarct of lung; septicemia
5	131 cholesterol feedings	10	Killed	0	0	None
6	Control	10	Killed	0	0	Chronic diffuse nephritis

TABLE 3.—*Observations in Litter 8, Inoculated with Strain of Streptococci from Coronary Thrombus, Grown in Aorta Medium, and Given Subcutaneous Implantation of Aorta Tissue*

Rabbit	Procedure	Duration, Mo.	Fate	Aortic Lesions	Lesions of Smaller Arteries	Pathologic Changes
1	Aorta implanted; 7 inoculations with streptococci	7	Killed	0	±	Round cell accumulations in heart
2	Aorta implanted; 7 inoculations with streptococci; 50 cholesterol feedings	7	Killed	+	+	Atheromatous ulcers of aorta; sclerosis of smaller arteries with thrombosis of one in lung
3	7 inoculations with streptococci; 50 cholesterol feedings	7	Killed	0	+	Sclerosis of coronary artery and of arteries in lung
4	50 cholesterol feedings	7	Killed	0	±	Lipoid infiltration of intima of small coronary branch
5	Control	2	Died	0	+	Nephritis; sclerosis of small arteries

Series 4, Inoculated with Hemolytic Streptococci from Sinuses.—Two cultures of hemolytic streptococci from the sphenoid sinuses of a patient with arthritis and chronic sinusitis were obtained for study and both typed out according to Holman's classification as Streptococcus anginosus. In an effort to determine whether sinus infection might cause arterial changes, a series of inoculations was started with these organisms.

Litter 9 consisted of seven rabbits of which rabbits 1 to 4 were inoculated ten times each with a sinus streptococcus over a period of about six months. Rabbit 5 received inoculations and cholesterol feedings. Rabbits 6 and 7 were saved as controls (table 4).

These inoculations with hemolytic streptococci were attended by arterial changes. Rabbits 1, 2 and 5 exhibited gross lesions of the aorta. No. 1 had small, raised atheromatous plaques. Microscopic examination of the lung disclosed intimal proliferation with deposition of fibrin in some of the smaller arteries (fig. 7). In places, arteritis was more active. Collections of fibrin and leukocytes were attached to the roughened intima and invaded the wall of the blood vessel. Pneumonia was not present.

The ascending aorta of rabbit 2 showed several whitish plaques. Microscopically there was patchy intimal thickening with dense infiltration by fibroblasts. Small

Fig. 5.—Photomicrograph of the wall of an artery of the lung in rabbit 4, litter 6, inoculated with Streptococcus viridans from a coronary thrombus and showing acute arteritis with thrombus formation: *I*, the intima, infiltrated with leukocytes; *M*, the media, showing fibrosis and hyaline change; *A*, the adventitia, densely infiltrated with polymorphonuclear leukocytes; *T*, the thrombus in the lumen.

areas of scarring were noted in the intima and media. In the myocardium, round cell infiltrations occurred. In the kidney, the arteries had thickened walls, often with complete obliteration of the lumina owing to proliferation of faintly stained cells that appeared to be endothelial. Many hyalinized arterioles were found. In all the arteries there was intimal thickening, usually with hyalinization of the

entire wall. In one arteriole, the wall contained a compact infiltration of mononuclear cells. In the lung, the arteries had thickened walls, with scarring of the media and roughening of the intima, on which fibrin was deposited.

Rabbit 5 lived four months, and at autopsy had large, whitish plaques in the aorta, confirmed by microscopic examination. In rabbit 3, some pulmonary arteries showed crescentic thickening of the intima. In rabbit 4, also, there was intimal thickening of the arteries of the lung.

Litter 10 consisted of six rabbits. The first four were inoculated with a hemolytic streptococcus, rabbits 5 and 6 being saved as controls. Only one

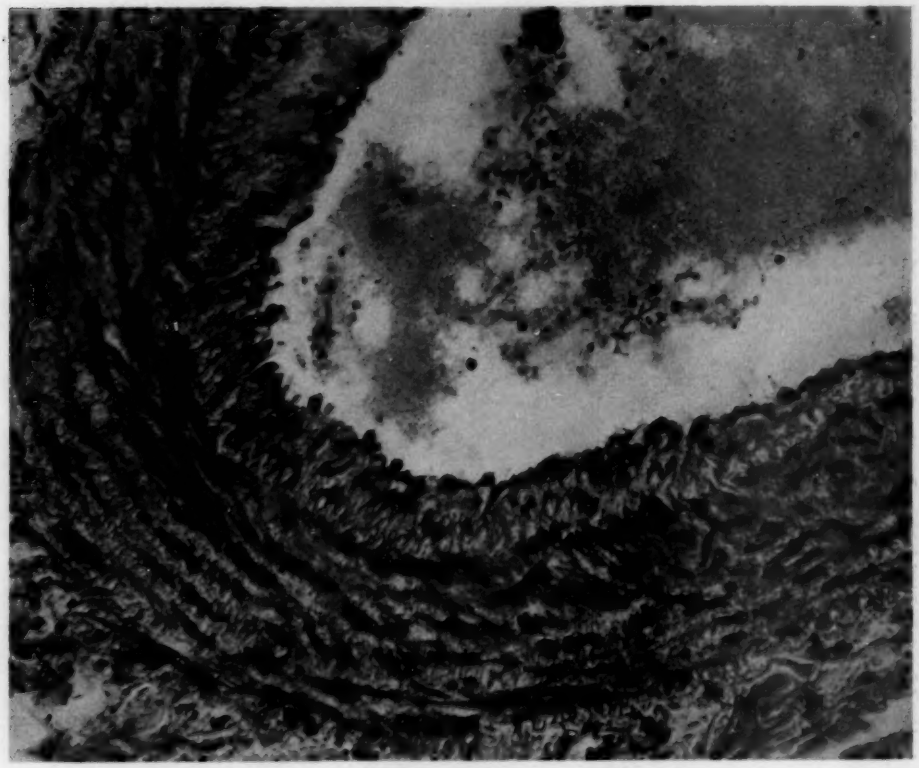


Fig. 6.—Photomicrograph of an artery of rabbit 4, litter 7, showing moderate thickening of the intima in early arteriosclerosis following inoculations with Streptococcus viridans from a coronary thrombus.

inoculated rabbit of this litter lived over one month. The others, and even one of the two controls, succumbed to pneumonia.

Rabbit 3, at autopsy, showed small, yellowish, indurated atheromatous ulcers of the ascending aorta. Microscopic examination of the lung disclosed irregular intimal thickening in medium-sized arteries. In one artery, the intima was greatly thickened and densely infiltrated by mononuclear cells having irregular or spindle-shaped nuclei. The underlying media had undergone hyalinization and lipoid infiltration. In the myocardium there were focal accumulations of lymphocytes.

Microscopic examination of rabbit 1 showed multiple myocardial abscesses, pneumonia and acute diffuse nephritis, with a hyaline change in the arteries of the kidney. Rabbit 2 died of pneumonia, and microscopic examination showed acute arteritis in the lung.

INOCULATION OF MONKEYS

Series 5.—Twelve monkeys (Macacus rhesus) were given a diet of vegetables, bread, milk, grains and nuts. One control was killed at the beginning of the

TABLE 4.—*Observations in Litter 9, Inoculated with Strain of Streptococcus Anginosus from Sphenoid Sinus*

Rabbit	Procedure	Duration, Mo.	Fate	Aortic Lesions	Lesions of Smaller Arteries	Pathologic Changes
1	10 inoculations with streptococci	6	Killed	+	+	Purulent arteritis in lung; atheromas of aorta
2	10 inoculations with streptococci	6	Died	+	+	Pneumonia and nephritis; arteritis in lung
3	10 inoculations with streptococci	9	Killed	—	+	Sclerosis of arteries of lung
4	10 inoculations with streptococci	9	Killed	0	0	None
5	10 inoculations with streptococci; 101 chlesterol feedings	4	Died	+	0	Atheroma of aorta
6	Control	9	Killed	0	0	None
7	Control	9	Killed	0	0	Interstitial nephritis

TABLE 5.—*Observations in Litter 10, Inoculated with Strain of Streptococcus Anginosus from Sphenoid Sinus*

Rabbit	Procedure	Duration, Mo.	Fate	Aortic Lesions	Lesions of Smaller Arteries	Pathologic Changes
1	5 inoculations with streptococci	1	Died	0	+	Multiple abscesses of myocardium; purulent arteritis in lung
2	5 inoculations with streptococci	1	Died	0	+	Pneumonia; acute arteritis in lung
3	6 inoculations with streptococci	7	Killed	+	+	Small, indurated atheromatous ulcers of aorta; sclerosis of arteries of lung
4	5 inoculations with streptococci	½	Died	0	0	Pneumonia
5	Control	½	Died	0	0	Pneumonia
6	Control	7	Killed	0	0	None

experiment for histologic preservation of the tissues and at the end of the experiment comparison was made with the two controls on which autopsies were made one year later. Eight monkeys were inoculated twenty-four times with streptococci (twice washed saline suspensions containing two billion bacteria per cubic centimeter) in a period of six months. Each animal was inoculated just below Poupart's ligament in the immediate vicinity of the right femoral artery. The initial dose of 1 cc. was gradually increased in five months to a final dose of 16 cc. At the end of six months the bacterial inoculations were discontinued, and the animals were permitted to live unmolested for another six months; then they were all killed and examined grossly and microscopically. Two controls were allowed to live with the inoculated animals. Monkeys 1 to 4 had been inoculated with a

succession of different strains from various cultures of streptococci, so that reinfection with a new strain had occurred from time to time. In monkeys 1 and 2, hemolytic S. anginosus, and in monkeys 3 and 4 nonhemolytic S. mitis, had been used. Monkeys 5 to 8 had received pure cultures; in monkeys 5 and 6, we used a strain of hemolytic S. anginosus from a patient with mastoiditis and extensive sinus thrombosis, and in monkeys 7 and 8 a strain of nonhemolytic S. mitis from the antrum of a patient with chronic sinusitis. In general, the animals remained in good health during the entire year of observation, but monkeys 7 and 8 acquired large inguinal abscesses, which healed spontaneously, and monkey 6

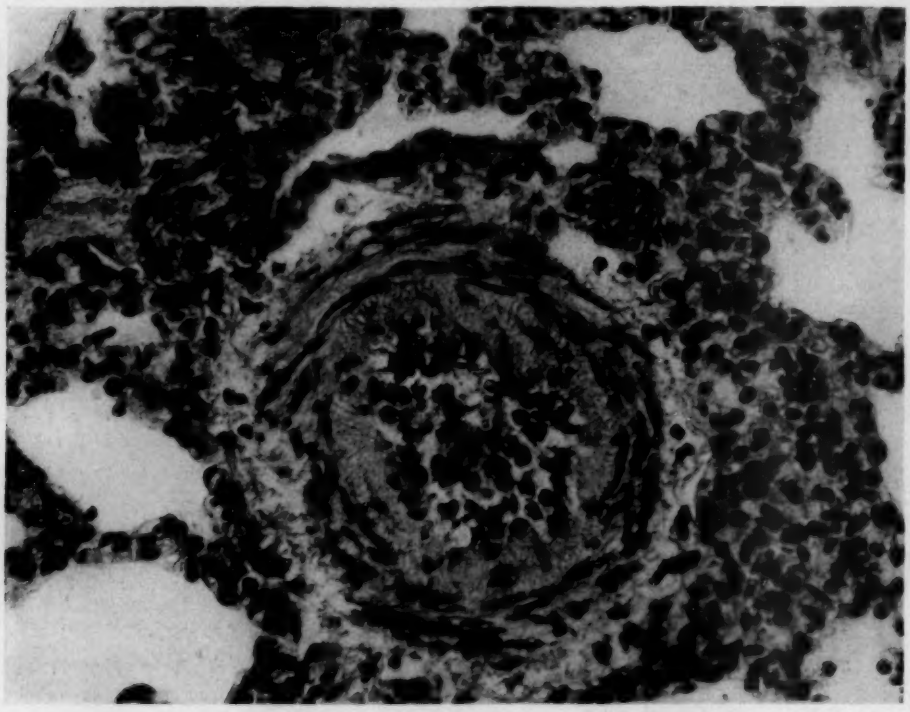


Fig. 7.—Photomicrograph of a small artery in a lung of rabbit 1, litter 9, showing acute purulent arteritis following inoculations with Streptococcus anginosus from a sphenoid sinus. There is purulent exudate in the lumen, infiltrating into the thickened intima. Note the loose fibrillar stroma in the adventitia.

a femoral hematoma and later a prolapsed rectum. All of our animals seemed to tolerate the inoculations without serious sequelae.

The monkeys after death showed no arteriosclerosis on careful gross examination. The changes observed in the various viscera were almost entirely microscopic, with the exception of fatty changes in some of the livers, which could be seen grossly. The microscopic changes included thickening of the walls of the pancreatic arteries in monkey 1. The pulmonary arteries in monkeys 3 and 4 had a lipoid infiltration and thickening of the intima, hypertrophy of the media and perivascular fibrosis. In monkey 5, the right femoral artery was thickened and surrounded by hyalinized muscle. In monkey 6, the right femoral artery

contained thickened intima and was surrounded by much scar tissue with obliterative arteriolitis in the surrounding tissue. The larger arteries of the kidneys contained thickened intimal linings. Monkey 7 showed a slight scarring around the right femoral artery. In addition to the arterial changes, monkey 1 presented accumulations of lymphocytes in the myocardium, subendocardium and subpericardium. Hyaline degeneration of the myocardium was observed about some of these foci. Similar changes were found in the hearts of monkeys 2, 6, 7 and 8. In the livers of monkeys 1, 2 and 6 there were marked accumulations of round cells in the periportal spaces, and in monkeys 2, 4, 6 and 8 there were focal and diffuse infiltrations into the interstitial tissue of the kidneys by lymphocytes.

COMMENT

It is unnecessary to review here the various attempts at experimental arteriosclerosis in animals. The important bibliography up to 1926 is included in a review by one of us (Benson).[1] The credit for first describing aortic lesions of rheumatic fever belongs to Klotz.[2] In 1926, von Glahn and Pappenheimer[3] made an exhaustive study of the changes in the aorta and other arteries in rheumatic fever. In a more recent publication[4] Pappenheimer and von Glahn described the intimal changes in a coronary artery, including a loose proliferation of endothelial tissue and the presence of an elevated parietal thrombus projecting into the lumen. Clawson[5] injected into rabbits and monkeys S. viridans from the blood of patients with rheumatic fever and subacute bacterial endocarditis, and obtained arteritis involving all coats. He considered this similar to the rheumatic lesions in man described by Klotz and by Pappenheimer and von Glahn.

Our observations on the aortic and other arterial changes bear a striking similarity to the descriptions that Clawson gave of his experimental lesions. Possibly, after all, there is a relationship between our experimental lesions, those described by Clawson, the rheumatic arteritis of Klotz and of Pappenheimer and von Glahn and the arteriosclerosis in the girl, 14 years old, referred to in our opening paragraph.

We have observed various stages of sclerosis in the aortas of inoculated rabbits in our series. The aorta shown in figures 4 and 8 had thickened intimal plaques composed of loose tissue containing irregular cells with deeply stained nuclei widely separated by fibrillar stroma

1. Benson, R. L.: The Present Status of Coronary Arterial Disease, Arch. Path. **2**:876, 1926.

2. Klotz, O.: Rheumatic Fever and the Arteries, Tr. A. Am. Physicians **27**:181, 1912.

3. von Glahn, W. C., and Pappenheimer, A. M.: Specific Lesions of the Peripheral Blood Vessels in Rheumatism, Am. J. Path. **2**:235, 1926.

4. Pappenheimer, A. M., and von Glahn, W. C.: Studies in the Pathology of Rheumatic Fever, Am. J. Path. **3**:583, 1927.

5. Clawson, B. J.: Experimental Rheumatic Arteritis, Arch. Path. **6**:947, 1928.

having scattered clefts. A comparison of these photomicrographs with those of Pappenheimer and von Glahn [6] disclosed a close similarity. The intima of our aorta was comparable with theirs, but the media of ours was only slightly changed as compared with theirs. The aorta shown in figures 1 and 2 represented a more advanced stage of arteriosclerosis. The loose tissue of the intima was being replaced by fibrous

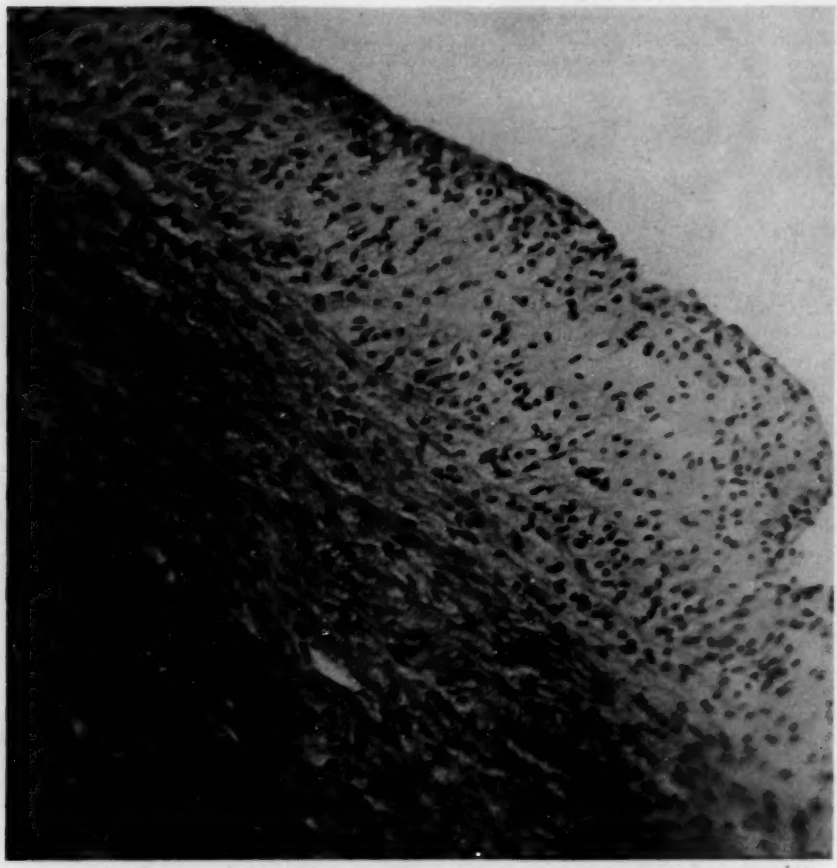


Fig. 8.—Photomicrograph of the aorta of rabbit 3, litter 6, showing irregular intimal thickening in arteriosclerosis following inoculations with Streptococcus viridans from a coronary thrombus.

tissue, with resulting scar formation. Fibroblasts were numerous, and deeply blue-staining fibrillar strands traversed the scarred intima. The media exhibited numerous irregular and spindle-shaped, deeply staining

6. Pappenheimer and von Glahn (footnote 4, plate 150, *A* and *B*, following p. 594).

cells, with hyaline change in the intervening tissue of the wall. The two aortas just referred to afforded instances of moderately early and fairly advanced stages of arteriosclerosis, respectively. Our other specimens varied chiefly in the extent of involvement and the stage reached.

Acute arteritis, sometimes purulent, occurred in some of the arteries. Figure 7 shows such a change in a small artery of the lung. The intima

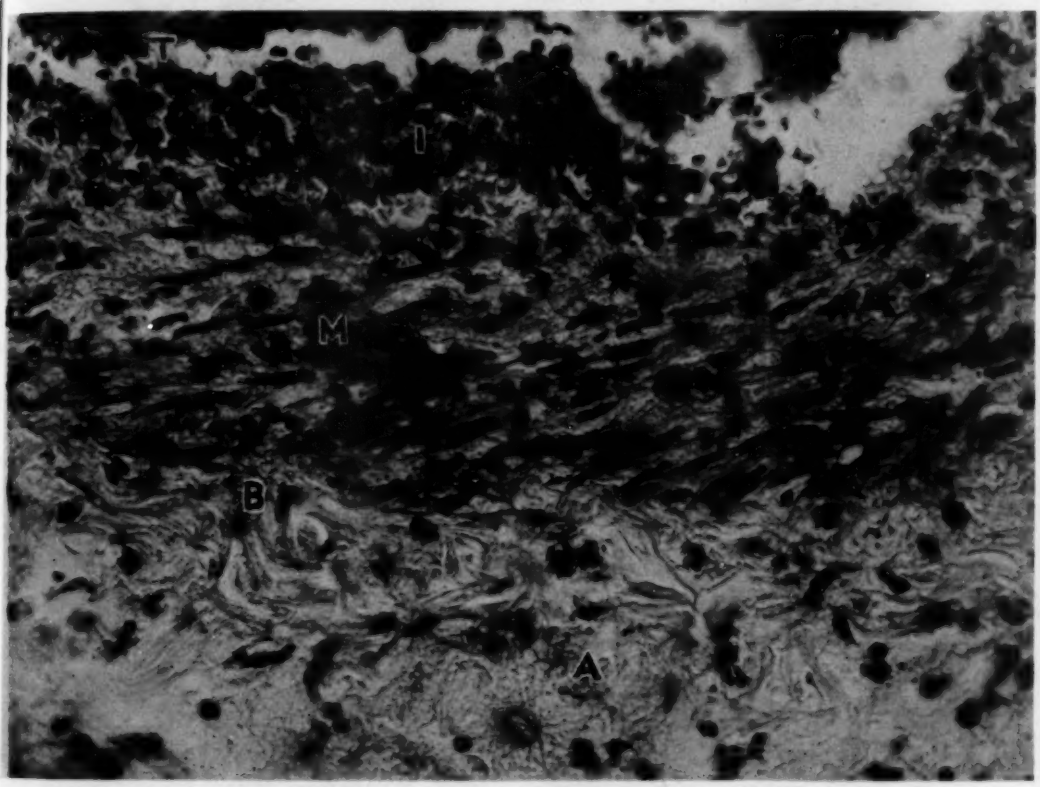


Fig. 9.—Photomicrograph of the wall of an artery in the lung, showing acute arteritis: *A*, the adventitia; *M*, the media with pyknotic nuclei; *I*, the purulent intima; *B*, bacteria in the wall; *T*, a thrombus in the lumen.

was thick and swollen and had a roughened surface with polymorphonuclear leukocytes and mononuclear cells attached to it. A mass of leukocytes and fibrin nearly filled the lumen, and polymorphonuclears were present in small number in the arterial wall. The advenitia consisted of a loose, relatively noncellular, fibrillar substance.

Purulent arteritis with an occluding thrombus was found in a large artery of one lung (fig. 9). The intima was thickened and densely

infiltrated with polymorphonuclears and fibrin, and this purulent exudate merged inseparably into a thrombus. Leukocytes were scattered throughout the wall, being especially dense in the adventitia. Bacteria could be seen in various parts of the wall, mostly diplococci.

Various stages of arteriosclerosis occurred. Figure 6 represents an early retrogressive stage with moderate intimal thickening characterized by loose, relatively noncellular tissue. Other arteries exhibited later stages of sclerosis with thickening of the entire wall, and with hyalinization.

In a review of series 3 and 4, we find that each of twenty rabbits was repeatedly inoculated with 1 cc. of streptococcus broth cultures, and that fourteen (70 per cent) acquired arterial lesions, of which those in seven were very definite on gross examination of the aorta. Thirteen of the twenty animals inoculated were fed on a normal diet, and eight of these (61 per cent) acquired arterial lesions. Nonhemolytic streptococci produced arteriosclerosis within three months in one rabbit, after twenty-three inoculations. The majority of the rabbits, however, were given twenty-seven inoculations each and were not examined until eight months had elapsed. Hemolytic streptococci were more virulent and killed most of litter 10 within one month. These animals had only five inoculations each, but showed abundant arterial changes. In litter 9 many survived ten inoculations and lived for nine months. Nearly all of the rabbits in this litter showed arteriosclerosis at the end of the experiment.

In the absence of gross aortic and arterial changes in series 5, we believe that our microscopic observations in monkeys are not conclusive.

Influence of Cholesterol.—The ingestion of an adequate amount of cholesterol for a sufficient length of time will produce arteriosclerosis in rabbits. Scarff [7] fed 50 Gm. of cholesterol, subdivided into daily doses of from 0.2 to 0.7 Gm., for six months before aortic changes appeared. Shapiro [8] found that thyroidectomy, splenectomy and gonadectomy facilitated experimental cholesterol arteriosclerosis.

We gave cholesterol feedings alone to one rabbit in all litters but one, and found no gross changes in the aorta after feeding a total of 65 Gm. of cholesterol over a period of nine months. In one rabbit, however, microscopic thickening of the arteries developed in seven months. From these findings we concluded that the quantity of cholesterol was not sufficient to create gross changes in the aorta. But when

7. Scarff, R. W.: The Production of Experimental Atheroma with Cholesterol, J. Path. & Bact. **30**:647, 1927.

8. Shapiro, B.: The Influence of Thyroidectomy, Splenectomy, Gonadectomy, and Suprarenalectomy upon the Development of Experimental Atherosclerosis in Rabbits, J. Exper. Med. **45**:595, 1927.

the same quantity was fed to litter mates that received intravenous injections of streptococci, there was a marked involvement of the blood vessels. By combined bacterial injections and cholesterol feedings, arterial lesions were produced in six of seven rabbits (85 per cent) and gross signs of arteriosclerosis in the aortas of four of the seven (57 per cent). Arteriosclerosis was more prominent in these rabbits than in those without hypercholesteremia. These rabbits received an average of only 46 Gm. of cholesterol during an average period of seven months. The minimum number of inoculations with streptococci was five and the maximum number twenty-seven. It is probable that infection of the blood stream plays an important rôle in precipitating cholesterol in the inflamed walls of blood vessels.

Spontaneous Arteriosclerosis in Rabbits.—Sufficient evidence has accumulated in recent years to show that spontaneous arteriosclerosis, primary in the intima, is not common in normal rabbits. Newburgh and Clarkson [9] reviewed this subject and differentiated between true arteriosclerotic lesions of the intima and the spontaneous medial calcifications. Dominguez,[10] in 1928, summarized all of the available literature on spontaneous arteriosclerosis, and expressed the belief that the unusual incidence reported by Miles in 1907 was a local phenomenon. Since that time many normal animals have been examined in this country and in Europe, and in an analysis of the records of more than thirty-five hundred rabbits examined and reported on in the last twenty years, Dominguez found that by including all arterial changes the incidence was only 6 per cent, and of these only 4 per cent, or 0.3 per cent of the total number, showed severe lesions. Nuzum and co-workers [11] reviewed this subject in 1930 and studied one hundred and ninety rabbits living under normal conditions for two or three years. They concluded that spontaneous arteriosclerosis is infrequent.

All of our controls and inoculated rabbits were litter mates, and none of the controls had arterial lesions that were visible grossly. In the microscopic examinations, which were as thorough in the controls as in the inoculated animals, we found a spontaneously thickened artery in a control only once. Within two months this animal died of pneumonia and nephritis. The fact should be emphasized that our experimental animals all belonged to young, healthy litters and ranged between two and three pounds (907 and 1,360 Gm.) in weight at the beginning

9. Newburgh, L. H., and Clarkson, S.: The Production of Atherosclerosis in Rabbits by Feeding Diets Rich in Meat, Arch. Int. Med. **31**:653, 1923.

10. Dominguez, R.: Effect on the Blood Pressure of the Rabbit of Arteriosclerosis and Nephritis Caused by Uranium, Arch. Path. **5**:577, 1928.

11. Nuzum, F. R.; Elliot, A. H.; Evans, R. D., and Priest, Blanche V.: The Occurrence and Nature of Spontaneous Arteriosclerosis and Nephritis in the Rabbit, Arch. Path. **10**:697, 1930.

of the experiments. All rabbits were examined by the end of ten months. In addition to our controls, we have examined more than one hundred uninoculated rabbits, exclusive of the present series, and have failed to find gross aortic lesions. We are convinced that spontaneous arterial lesions are rare in young healthy rabbits on a normal diet.

SUMMARY

We have described lesions in the aorta and other arteries in rabbits inoculated with streptococci from sclerotic coronary arteries, coronary thrombi and sinusitis. These lesions include acute arteritis with or without thrombosis and arteriosclerosis of the aorta and other arteries. The experimental changes are comparable with the corresponding arterial diseases found in man. Slight arterial changes were observed in monkeys following injections of streptococci, but the changes are regarded as inconclusive.

HYPERVITAMINOSIS D AND ARTERIOSCLEROSIS*

H. L. VANDERVEER, M.D.
TORONTO, CANADA

Much experimental work has been done to determine the toxic effects on laboratory animals of excessive doses of viosterol (irradiated ergosterol). It has been shown that massive doses produce a marked metabolic disturbance characterized by a condition of hypercalcemia and hyperphosphatemia, and result in the formation of calcium deposits in various organs of the body. The recent work on viosterol has been summarized by Pfannenstiel.[1]

Ergosterol, the provitamin D, is a sterol obtained principally from yeast and ergot, and, when irradiated, has an antirachitic property 2,000 times that of cholesterol, in which substance ergosterol is found as an impurity (Blunt and Cowan[2]). Koch, Koch and Ragins[3] concluded, after experimenting with various sterols, that the provitamin D activity is not limited to ergosterol. The toxic effects of massive doses of viosterol have been reported on by Blunt and Cowan,[2] Duguid,[4] Spies and Glover,[5] Shohl, Goldblatt and Brown,[6] Klein[7] and others. Laas[8] in his experiments on rabbits gave single massive doses of viosterol of high potency, and observed vitamin D sclerosis of the aorta as early as the fifth day of the experiment. The altered phosphorus and calcium metabolism of animals treated with viosterol has been reported by Ashford,[9] Watchorn,[10] Brown and Shohl[11] and others. Rabl[12] found that sodium phosphate increased the toxicity of viosterol. The possibility has been suggested that vitamin D promotes calcification in

* Submitted for publication, July 20, 1931.

* From the Department of Pathology and Bacteriology, University of Toronto.

1. Pfannenstiel, W.: Lancet **2**:845, 1928.

2. Blunt, K., and Cowan, R.: Irradiated Foods and Irradiated Ergosterol, J. A. M. A. **93**:1301, 1929.

3. Koch, F. C.; Koch, E., and Ragins, I. K.: J. Biol. Chem. **85**:141, 1929.

4. Duguid, J. B.: J. Path. & Bact. **33**:697, 1930.

5. Spies, I. D., and Glover, E. C.: Am. J. Path. **6**:485, 1930.

6. Shohl, A. T.; Goldblatt, H., and Brown, H. B.: J. Clin. Investigation **7**: 505, 1930.

7. Klein, I. J.: Effects of Massive Doses of Irradiated Ergosterol; Preliminary Report, J. A. M. A. **92**:621, 1929.

8. Laas, E.: Virchows Arch. f. path. Anat. **278**:346, 1930.

9. Ashford, C. A.: Biochem. J. **24**:661, 1930.

10. Watchorn, E.: Biochem. J. **24**:631, 1930.

11. Brown, H. B., and Shohl, A. T.: J. Biol. Chem. **86**:245, 1930.

12. Rabl, C. R. H.: Deutsche med. Wchnschr. **55**:63, 1929.

the body through a stimulative action on the parathyroid glands. It is notable that the toxic symptoms and the sites of deposition of calcium in tissues of dogs following treatment with parathyroid extract described by Learner [13] and Hueper [14] are similar to those in animals as a result of hypervitaminosis D. Morgan and Garrison [15] studied the effect of vitamin D and the reaction of the diet on the response to parathyroid extract, and concluded that vitamin D had an intensifying effect on the toxic action of parathyroid extract, also that vitamin D and an alkaline reaction of the diet, together or separately, increase the response to parathyroid extract. Pappenheimer [16] stated that cod liver oil and viosterol in therapeutic doses are both antirachitic in the absence of parathyroid gland or of thymus, or of both. The purpose of this study has been to produce arteriosclerosis in rabbits by means of large doses of irradiated ergosterol, and to follow the sequence of events in the pathologic processes in the affected vessels; and also to ascertain the order of involvement of tissues other than arteries that shown evidence of calcareous degeneration as a result of hypervitaminosis D.

THE EFFECT ON RABBITS OF THE ADMINISTRATION OF TOXIC DOSES OF IRRADIATED ERGOSTEROL

Materials and Methods Used.—For these experiments twenty-four young healthy rabbits were selected, ranging in age from 3 months to 1 year. For treatment with irradiated ergosterol, these were divided into small groups according to their age and weight. The irradiated ergosterol used was a biologically tested product with a potency of 10,000 D; [17] therefore, the vitamin D potency was 10,000 times the vitamin D potency of good cod liver oil. The dosage selected for the year old rabbits was approximately 0.5 cc. per kilogram of body weight, and the dosage for the younger animals was varied proportionately. In all the experiments the irradiated ergosterol was given in biweekly doses by the subcutaneous route, sterile precautions being used, and the termination of each experiment was by the spontaneous death of the treated animal. At autopsy, after macroscopic examination, blocks of tissue from the following organs were immediately preserved in a neutral solution of formaldehyde, U. S. P. (1:10), and in Zenker's solution: aorta (complete) and branches, heart, kidneys, liver, lung, stomach, spleen, pancreas, suprarenal glands, brain, thyroid gland, parathyroid and salivary glands and bone marrow of the femur. After fixation, frozen and paraffin sections were made. Frozen sections were stained with sudan III to demonstrate fat, and counterstained with hematoxylin. The principal stains used for the paraffin sections were hema-

13. Learner, A.: J. Lab. & Clin. Med. **14**:921, 1929.

14. Hueper, W.: Metastatic Calcifications in the Organs of the Dog After Injections of Parathyroid Extract, Arch. Path. **3**:14, 1927.

15. Morgan, A. F., and Garrison, E. A.: J. Biol. Chem. **85**:687, 1930.

16. Pappenheimer, A. M.: J. Exper. Med. **52**:805, 1930.

17. Viosterol 10,000 D was supplied for the experimental work by Mead, Johnson and Company.

toxylin and eosin, Verhoeff's method for staining elastic tissue, van Gieson's picrofuchsin, alizarin and von Kossa's silver nitrate method for identifying calcium deposits.

Macroscopic Observations.—The general effects noted in the rabbits receiving large doses of irradiated ergosterol were a continuous loss of weight and finally cachexia followed by death. At autopsy, there was no gross lesion indicating the cause of death; the animals were poorly nourished, and there was a noticeable absence of adipose tissue.

The aorta, when marked medial involvement was present, showed an irregularity of contour from an outpouching and twisting of its walls. Such an aorta, when cut open, revealed a multitude of lesions that were visible on its intimal surface as grayish, plaquelike depressions varying in size and bounded by the normal arterial wall, which had the appearance of a fold in this situation. The findings varied in the different animals, from a minor degree of arterial involvement situated about the aortic sinuses, to a diffuse embedding of the calcareous deposits in the aortic wall and its immediate branches.

The heart in the longer experiments was generally hypertrophied—in all cases the walls were relaxed and flabby. Except in one animal, the cut surface always appeared normal. In this case (no. 84) the heart, when cut open, showed a grayish-white area, irregular in outline, which extended through the interventricular septum. Later this proved to be an area of calcification. In two cases (nos. 84 and 182), the anterior surface of the pericardium had a wrinkled, withered appearance, and this portion was shown in stained sections to have undergone a hyaline change.

The kidneys were normal in shape; occasionally they were congested and soft. The capsules stripped readily, leaving a glistening, smooth surface. The cut surfaces of these kidneys presented no gross abnormalities; only occasionally a minute area of calcification was visible.

In a few animals a pneumonic condition was evident in the lungs. Frequently the lower borders of the lungs were congested. In one case (no. 182), a section of the pleural surface appeared similar to the pericardial surface mentioned—dry and wrinkled, and somewhat rough to the touch. Microscopically, this part of the membrane showed a calcium deposit covering its surface, while in the adjoining sections a hyaline change had occurred.

The liver was usually normal in appearance, unless the rabbit was infected with coccidia. The liver of one animal (no. 138) had an entire lobe calcified; this lobe was attached to the normal-appearing liver by a firm, fibrous pedicle.

The spleens of these animals were, as a rule, much smaller than normal and appeared congested.

The bone marrow of the femur was soft and varied from pale to deep red.

The stomach in one animal had a firm, raised, yellowish-white area in its wall; this region was later shown to contain calcium. No other abnormalities were noted.

The other organs examined appeared normal.

Microscopic Observations in Arterial Lesions.—The type of arteriosclerosis found in the arteries of rabbits treated with toxic doses of irradiated ergosterol is comparable to that produced in rabbits by the administration of epinephrine hydrochloride, barium chloride, digitalin and diphtheria toxin, as described by Klotz,[18] and to the experimental work-arteriosclerosis described by the same author,[19] which resembled the Mönckeberg sclerosis of the peripheral arteries in human beings, the main points of difference between vitamin D sclerosis and the Mönckeberg type being the site of the earliest lesions, which will be described later. There is a general agreement among experimenters as to the type and histologic characteristics of the advanced vitamin D lesion, but more differences of opinion are expressed about the earliest site of arterial involvement. The variation in the early observations reported by different authors is possibly due partly to the fact that individual animals of the same species vary in their susceptibility to the toxic action of viosterol, and to the variation in potency of the preparations of irradiated ergosterol used in their experiments (Hess, Lewis and Rivkin [20]).

In describing arterial lesions found in rabbits it is well to keep in mind the fact that this animal is subject to spontaneous sclerosis, which may attack both the intimal and the medial coats of the blood vessel, and which manifests itself as a proliferative lesion in the intima, and a degenerative one in the media (Nuzum, Elliot, Evans and Priest [21]). The medial lesions occurring in rabbits treated with viosterol differ from the Mönckeberg type of arteriosclerosis both in the fact that the muscle cells are not the earliest site of the calcareous deposit in these arteries, and in the fact that the middle zone of the media is not the area commonly involved in the mature lesion. The earliest calcareous deposits are intimately related to the elastic fibers in the aorta and to the internal elastic lamina of the muscular type of arteries, and in both types of vessel to the interstitial substance adjacent to the involved fibers. The location of the early lesions in the aorta tends

18. Klotz, Oskar: Brit. M. J. **2**:1767, 1906.

19. Klotz, Oskar: Montreal M. J. **37**:165, 1908.

20. Hess, A. F.; Lewis, J. M., and Rivkin, H.: Newer Aspects of Therapeutics of Viosterol (Irradiated Ergosterol), J. A. M. A. **94**:1885, 1930.

21. Nuzum, F. R.; Elliot, A. H.; Evans, R. D., and Priest, B. V.: The Occurrence and Nature of Spontaneous Arteriosclerosis and Nephritis in the Rabbit, Arch. Path. **10**:697, 1930.

to be along the inner half or inner third of the arterial wall; in the arch, calcareous plaques are frequently scattered throughout the medial coat, while on the lower section of the abdominal aorta the lesions lie to the inner third of the media, where, without exception, they are found in early lesions of the muscular type of arteries. In the experiments concerned here, only in one artery was evidence found of a secondary reaction, proliferative in nature, occurring in the intima over an underlying area of medial degeneration. Intimal proliferation was more common in relation to a hypertrophied section of the media in which no other pathologic change was demonstrable. In the late stage, the medial lesions were degenerative, causing such destruction to the medial coat that at the site of extensive calcification this coat appeared markedly thinned, while externally the vessel showed small

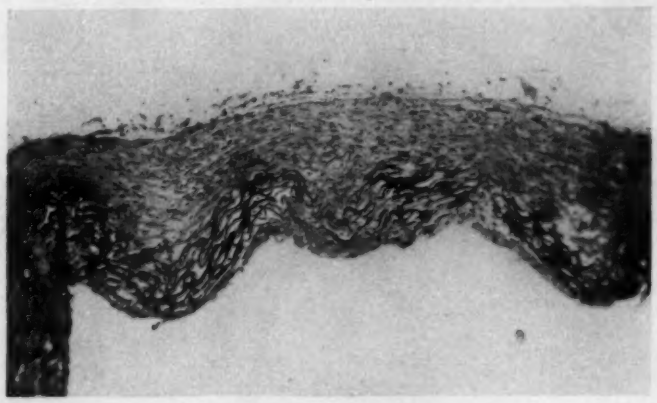


Fig. 1 (experiment 356).—Arch of aorta showing vitamin D arteriosclerosis. Note calcareous deposits about elastic fibers in the media. Hematoxylin and eosin; × 80.

outpouchings of its wall. The explanation of these apparent dilations was found on microscopic examination of the cross-sections of the walls of the vessel. It was then noted that in these areas the elastic fibers were straightened out and rigidly fixed in an extended position by calcareous granules deposited over the elastic fibers and in the tissues about them; therefore, these portions of the wall of the vessel could not undergo the usual postmortem contraction. On the intimal surface, these lesions appeared as depressed areas varying in circumference from 0.1 to 0.4 cm., with their margins thrown up into a fold. The intima over them appeared unchanged.

In the aorta, the earliest calcareous deposits appeared focally in the cement substance along the surface of the elastic fibers of the media, or in the intercellular matrix lying immediately adjacent to these fibers, so that they first appeared to be outlined with a fine, granular

deposit, which later took the form of an incrustation. These granules stained a deep purple with hematoxylin, and a color varying from brown to black with silver nitrate, a finding that has been accepted in the past as representing a deposit of calcium salts in the tissues. Recently Cameron [22] reported that there is no specific stain for calcium, and that areas of calcification are very complex in structure, with calcium occurring only as a small portion of the deposit. Preceding the appearance of the granular deposit, a change took place in the intercellular matrix; there was an increase of this substance, produced apparently by some process of dilution, whereby its staining qualities

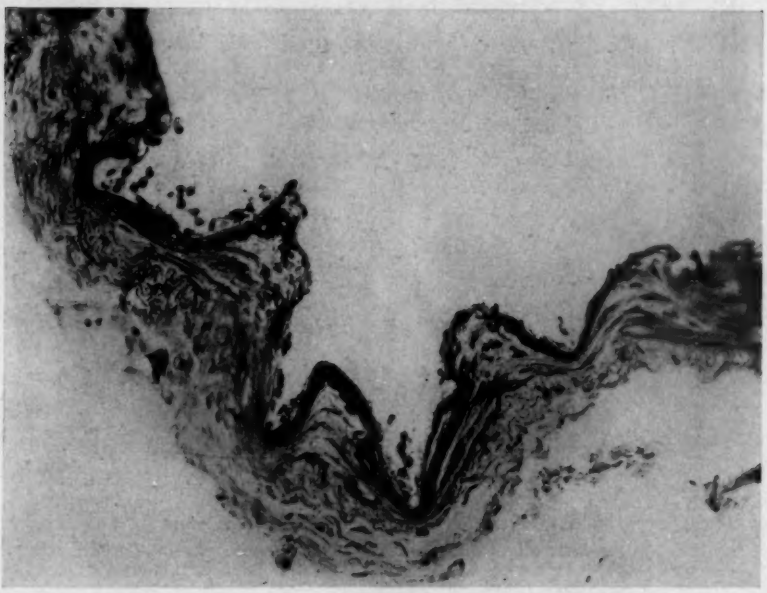


Fig. 2 (experiment 355).—Iliac artery showing calcareous deposit in the cement substance along the medial border of the internal elastic fiber. Hematoxylin and eosin; × 200.

were altered so that the areas stained faintly or not at all. This change was neither a mucoid nor a fatty degeneration.

The incrustation of short lengths of elastic fibers mechanically interfered with the pliability of these fibers, so that the contraction waves became coarse and irregular; adjacent affected fibers appeared to increase in length and tended to contract in unison and later straighten out and lie in parallel lines in the axis of the circumference of the media. Some of the fibers were frequently pushed widely apart in an uneven manner owing to the increase in the intercellular

22. Cameron, J. R.: J Path. & Bact. **33**:929, 1930.

substance and also to the contraction of the adjacent muscle fibers. Later there was evidence of a crowding together of the affected fibers away from the direction of the lumen of the vessel.

At this stage, the muscle cells lying between the involved elastic fibers still appeared healthy and stained normally, while the elastic fibers themselves were unaltered and stained evenly by Verhoeff's method. In more advanced lesions, the staining of the elastic fibers became interrupted at varying intervals and later the stained fibers presented a granular appearance. The muscle cells became compressed by the

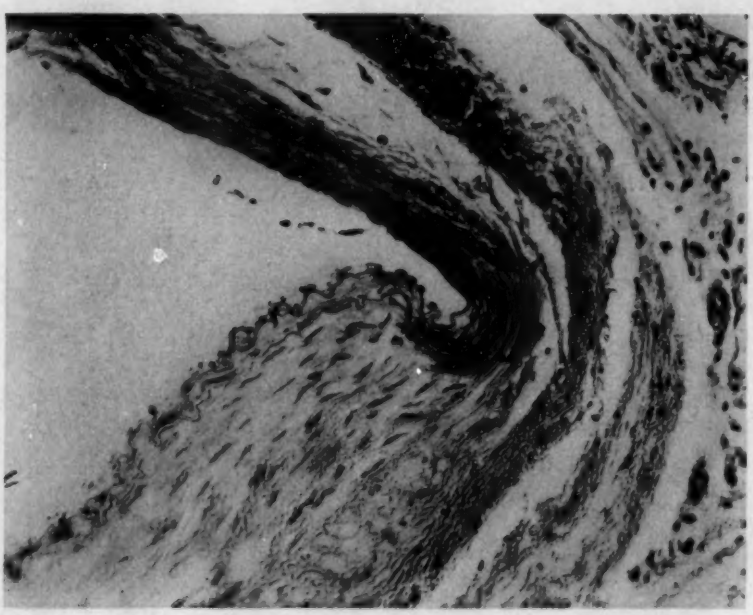


Fig. 3 (experiment 138).—Renal artery showing calcification of media and adventitia in one portion, and elastic laminae closely compacted. Hematoxylin and eosin; × 200.

crowding together of the elastic fibers, and the cytoplasm and nuclei of the thin, elongated cells stained deeply. Later, when a heavy, plaque-like deposit of calcium was formed in the interstitial substance, the muscle cells in the central part of the lesion underwent degeneration, the cytoplasm of each cell becoming swollen and vacuolated with a stringy appearance of the cell margin. The nuclei were small and pyknotic. Following this change, the muscle cells became necrotic, the elastic fibers atrophied and were broken up into small fragments, and a coarsely granular mass of calcareous material filled the area of degeneration. The margins of these lesions shaded off into normal tissue. It is surprising what an accumulation of calcareous material

is first deposited over and about the elastic fibers and muscle cells, before either show evidence of marked degeneration.

In the muscular arteries, the sequence of arteriosclerotic change was similar to that found in the elastic type of blood vessel. The earliest site of involvement was the medial side of the elastic lamina. Here a focal change occurred in the interstitial substance, revealed by an increase in its volume and a decrease in the intensity of its staining qualities. Accompanying this process, a fine, granular deposit of calcium was formed along the medial surface of the internal elastic lamina, at first as a thin covering, and later as a dense coat surrounding it for

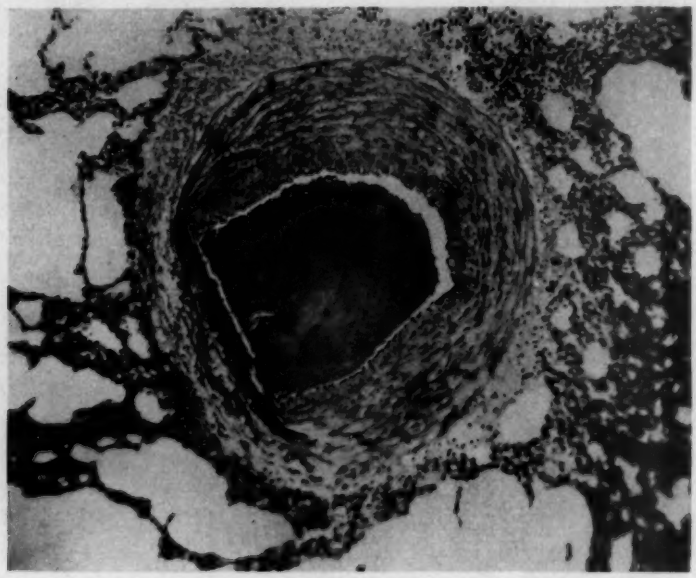


Fig. 4 (experiment 182).—Bronchial artery showing calcification of one segment of the media, while the remaining portion is hypertrophied. Hematoxylin and eosin; × 80.

short intervals. This deposit spread in the adjacent intercellular substance, until at a late stage the entire media of a segment of the wall of the vessel was involved, the adventitial coat rarely being included. None of the lesions in the muscular arteries showed evidence of a degenerative change in the internal elastic membrane. There was only a mechanical interference with its pliability, shown by a straightening out of the usual fine contraction waves so that the membrane was thrown into coarse, irregular folds when the underlying active muscle cells contracted. The muscle cells in these arteries were compressed from the direction of the lumen of the vessel outward, but except for

small areas showing a hyaline-like change where this compression was most marked, no primary degeneration of the muscle cells occurred. The cells lying just external to the internal elastic membrane were the first to be caught in the outward extension of the calcium deposit from the region of the lamina, and thus lost their contractile power and followed the uneven folds of the lamina, while the more external, healthy muscle fibers continued to contract in a normal manner.

The apparent thinning of the wall of the vessel in early vitamin D lesions before definite degeneration of the tissue elements takes place is a mechanical phenomenon due to pressure from within the lumen of the vessel outward, first manifested by the compression of the muscle cells in the layer situated directly external to the inner elastic lamina, and gradually involving the entire medial coat. Evidence that the inner coats are most affected by a process of stretching and loss of elasticity of their fibers is found in the aorta studded with vitamin D lesions which, when cut open, everts in both a longitudinal and a horizontal direction.

In this series of experiments, intimal lesions were uncommon as a result of hypervitaminosis D. The lesions found in the intima were minute and almost without exception hyperplastic, produced by a splitting of the internal elastic membrane and an increase of the muscle cells and fibrous tissue. In all these cases, the underlying arterial wall showed no evidence of degeneration or calcification, but instead, a marked hypertrophy of the muscle cells and an increase in the number of elastic fibers. These lesions suggested a compensatory hyperplasia of the intima produced by increased functional activity, the same stimulus as that affecting the cell activity of the underlying medial coat. Increased work over an extended period has been found to cause hypertrophy of the musculo-elastic layer of the intima and splitting of the internal elastic membrane in carotids of experimental animals (Klotz [23]). One animal showed a more advanced lesion of this type; in this, the internal elastic lamina was split into numerous fibers, which were spread apart by proliferating layers of muscle cells. In the resulting nodule formation, many of the muscle cells were rounded in outline with clear, vacuolated cytoplasm, containing centrally placed pyknotic nuclei. Varela [24] and his co-workers described this type of lesion as one specific for vitamin D sclerosis.

As mentioned before, in one animal only was there found a small regenerative lesion of the intima, which occurred over an area of extensive medial calcification. Duguid [4] from his experiments reported hyperplasia of the intima only over areas of medial degeneration.

23. Klotz, Oskar: J. Exper. Med. **12**:707, 1910.

24. Varela, B.; Collazo, J. A.; Moreau, J., and Rubino, P.: Virchows Arch. f. path. Anat. **274**:270, 1929.

Evidence of a toxic degeneration of the muscle cells occurred in focal areas in portions of the aorta where there was no suggestion of a calcareous lesion. The injured cells were from round to oval in outline, with clear, colorless cytoplasm and contained centrally placed, pyknotic nuclei. In frozen sections, these cells showed no evidence of fat. These lesions occurred in the outer or inner third of the medial coat or throughout the entire medial coat. Laas [8] found the same type of degeneration in rabbits exposed to chronic intoxications, particularly in animals that had died a natural death. In the present experiments, the same type of degeneration was found occurring in muscle cells situated in the longitudinal muscle layer of the stomach.

TABLE 1.—*Relation of Length of Experiment to Degree of Calcification of Aorta*

Experiment	Days	Gain or Loss in Weight, per Cent	Calcification of Aorta	Age of Rabbit, Mo.	Total Dosage of Viosterol 10,000 D, Cc.
353	18	—15.7	0	12	0.5
356	25	—31.9	+	12	1.1
354	28	—42.6	++++	12	3.6
355	33	—20.4	++++	12	4.6
352	42	—32.9	+	12	6.6
357*	70 (killed)	+ 6.1	0	12	7.1
139	14	—17.5	++++	12	2.0
178	30	—34.1	++++	12	8.0
138	41	?	++++	12	6.0
182	62	—37.2	++++	12	17.0
11	11	?	++++	3	2.0
9	16	?	0	3	2.5
10	25	—37.0	+++	3	3.5
12	25	—38.0	+++	3	3.5

* The animal in experiment 357 received calcium (in the form of calcium gluconate-Sandoz) in daily doses of 15.4 mg. intramuscularly. The animal gained in weight and was killed on the seventieth day of the experiment. No areas of calcification were found in the tissues.

The occurrence of the calcareous lesions in the inner thirds of the arteries has been referred to. The region of the aortic sinuses was the first area attacked. The process extended downward, and no segment of an artery within the involved area was found free. The process frequently stopped abruptly at the diaphragm, or extended along the abdominal aorta to its branches and to the vessels of the extremities. There was no evidence of grouping of the calcareous plaques about the ostea of the intercostal arteries or the lower branches of the aorta. There was a tendency for the lesions to miss the under part of the aortic arch and the posterior wall of both the thoracic and abdominal aorta. The most severe changes were in the arch and thoracic aorta. The cardiac valves and the pulmonary vein were frequently extensively calcified.

Fatty changes were not present in all lesions of medial calcification produced by viosterol. In frozen sections stained with sudan III it was usual to find from fine to coarse droplets of fat between the elastic fibers in the medial coat surrounding a thick, calcareous plaque. The droplets tended to mass in the tissues lying at the external margin of the calcified area. These observations were not constant, for often in an adjacent lesion of apparently equal intensity no fat could be demonstrated; nor was evidence of fat found in relation to the early granular deposits in the media. After decalcification of frozen sections, the site of a previously heavy calcareous deposit and the external border of such a lesion often showed fine droplets of pale yellow-staining fat, or coarse fat globules, located in the interstitial substance lying between

TABLE 2.—*The Effect of Excessive Doses of Viosterol on Rabbits*

Treatment	Experiment	Days	Total Dose, Cc.	Initial Weight Gm.	Weight Loss, per Cent	Aorta	Muscular Arteries	Heart	Lungs	Kidneys	Stomach	Liver
Viosteral	354	28	3.6	1,830	42.6	++++	++	+	0	+++	Trace	0
10,000 D,	178	30	8.0	2,480	34.1	++++	+	0	0	++	++++	0
biweekly	355	33	4.6	1,565	20.4	++++	+++	Trace	0	++	++	0
dose 0.1–1.0 cc.	182	62	17.0	2,150	37.2	++++	+++	0	++	Trace	0	0
Viosterol	139	14	2.0	2,000	17.5	++++	++	+	++	++++	+++	0
10,000 D,	356	25	1.1	985	31.9	+	0	0	0	Trace	0	0
biweekly	10	25	3.5	875	37.0	+++	0	0	0	++	+++	0
dose 0.1–0.5 cc.	12	25	3.5	810	38.0	+++	+	Trace	Trace	+	0	0
	138	41	6.0	2,000	?	++++	++++	0	0	+	++	+++
	352	42	6.6	940	32.9	+	0	0	0	0	0	0

+ = degree of calcification of tissues.

the elastic fibers. In the intima, fat droplets were occasionally present within the cells of a proliferative lesion.

Calcification in Organs Other Than Arteries.—An extensive deposit of calcium in the cardiac muscle occurred in one animal in an area of degenerated tissue surrounded by a zone of infiltrating leukocytes and walled off by fibrous tissue. In three other animals, minute deposits of calcium were found in small focal areas of degenerated muscle where the muscle fibers were vacuolated. In these four cases, the coronary arteries were moderately sclerosed. In two other cases there was a hyaline degeneration of a portion of the pericardium.

The kidneys of eight animals showed calcareous deposits in the parenchyma and slight deposits in some of the blood vessels. The lesions varied in severity; only in one case was there marked involvement of the organ. Calcium casts occurred, as a rule, in the tubules of the cortex and frequently were associated with further destruction

of the tubular epithelium in the areas where these casts had lodged. Calcium deposits were found in the basement membrane of scattered tubules in the cortex, also rarely in the glomerular capsules and in the media of the interlobular arteries and arterioles. In four of these cases, the renal artery was sclerosed. Sections of kidney stained for fat showed heavy fat deposits in the degenerating tubules that were filled with casts of calcareous material. In all the kidneys there was a granular degeneration of the tubules, generally accompanied by cloudy swelling.

Two animals showed calcium deposits in the lungs; in one of these there were a few scattered granular particles in the mucous membrane of a large bronchus; in the other (no. 182), the greater part of the pleura of one lobe, which was covered on its inner surface by an extensive inflammatory exudate, was completely calcified. Areas of the pleura not calcified had undergone hyaline degeneration. Calcification had also occurred in the elastic fibers in the wall of a large bronchus. The bronchial arteries, the pulmonary vein and the smaller vessels were markedly sclerosed. Both these animals had an inflammatory process in the lung simulating bronchopneumonia. No calcification occurred in the bronchial muscles in any of the animals.

Calcification occurred in the livers of three animals. In two, the process concerned greatly enlarged bile ducts, the result of infection with coccidia. The third animal had one lobe of the liver calcified, as mentioned before. Examination of the adjacent, fairly normal tissue leading to the pedicle of the calcified lobe showed marked destruction of the liver parenchyma and fibrosis, but no indication of whether the disturbance was a vascular or an inflammatory one. In every case some form of tissue degeneration was present in the liver. Congestion was generally marked, with varying degrees of atrophy of cells about the central areas.

In six animals, the stomach showed calcification of a portion of its wall; in four, moderate calcareous deposits were located in the circular muscular coat; in one, a small amount of calcium was deposited in the longitudinal muscle fibers, with a few scattered granules in the glands of the mucous coat; the remaining animal had a moderate deposit only in the serosa, and in this area there was a small sclerosed artery. These calcareous deposits in the muscle fibers were found only in regions of focal degeneration, which were scattered through the muscle coats. The cytoplasm of the muscle cells in these situations first appeared vacuolated; later a fine granular deposit of calcium was laid down either in the degenerating cells or in the intercellular substance about their borders.

Brain tissue showed no abnormalities and no sclerosis of the arteries.

The spleen in the majority of cases was markedly congested and frequently showed large amounts of hemosiderin phagocytosed by endothelial cells. The malpighian bodies were degenerated and atrophied, and diminished in number, so that often few corpuscles remained in the degenerated pulp tissue. In one case, the splenic artery was sclerosed.

No calcified areas were found in the suprarenal glands. In one gland a small, oval section of bone complete with a centrally placed medulla was incorporated in the cortex just beneath the capsule. There was no disturbance of the surrounding parenchyma.

The thyroid glands indicated various states of activity. Two were extensively fibrosed; the others appeared normal.

There were no gross abnormalities in the parathyroid glands; they were about normal in size or showed a very slight increase above the normal, except in one case in which the glands were definitely enlarged. The parenchymal cells were normal in size, shape and staining qualities and in relation to each other. In these parathyroid glands the capillary channels were distended and congested. In dogs dying of acute hyperparathyroidism, the parathyroid glands are reduced to one-half their size, and the parenchymatous cells are distorted and diminished in size (Jaffe and Bodansky [25]).

The salivary glands were normal. The pancreas in the majority of cases indicated no abnormality; however, in three cases the islet cells had undergone degeneration; the cytoplasm was granular and occasionally vacuolated, the cell margins were indefinite, and many of the nuclei were small and stained deeply.

The bone marrow in every case showed a marked hyperplasia of the erythrogenic centers, with congestion of the capillaries, usually accompanied by an increase in the number of granular calls. There was no evidence of destruction of marrow or of fibrosis such as that produced by experimental hyperparathyroidism (Jaffe and Bodansky [25]).

Rabbit 357 received a total dosage of viosterol that exceeded any other dose administered in the same experimental group, together with daily doses of calcium gluconate. I cannot give any explanation why this animal, when killed on the seventieth day of the experiment, showed no evidence of calcareous lesions either in the arteries or organs.

CONCLUSIONS

Vitamin D arteriosclerosis is not a specific type, for well developed lesions resemble those experimentally produced by the administration of epinephrine hydrochloride, barium chloride and digitalin, the main point of difference being the primary site of tissue involvement. Inflam-

25. Jaffe, K. L., and Bodansky, A.: J. Exper. Med. **52**:669, 1930.

matory processes are not a factor in its production, as no cellular infiltration occurs in or about these lesions. Nutritional disturbances alone do not account for their early formation, for instead of the entire arterial wall becoming involved, the lesions are patchy in their distribution and appear to follow the location of areas of stress and strain in the vessel. Evidently the toxic and mechanical factors are more important. Toxic degeneration occurs in these arteries, and though not always in relation to the primary medial calcification, it appears to have a relation to the changes in the intercellular substance that allow calcium to be deposited in that region. No specific type of degeneration of tissue precedes the initial deposit of calcium about the elastic fibers. At a later period, the mechanical factor introduces its local influence. Evidence occurs in the tissues of the failing ability of the arteries to maintain a proper circulation in the body. At autopsy, the heart is hypertrophied, there are passive congestion of the spleen and focal necrosis in the liver, congestion of the lungs and of the capillaries of the bone marrow, and generalized hypertrophy of the walls of the smaller blood vessels.

The first action of vitamin D on the tissues of organs other than blood vessels appears to be a toxic one, as shown by the focal areas of loosening of the muscle cells from their embedding substance, so that they tend to assume a rounded outline and later undergo degeneration and calcification. This is a prominent observation in the cardiac muscle and the muscle of the wall of the stomach. The early toxic action on the renal epithelium is also marked by an extensive granular degeneration and cloudy swelling. The calcium deposits in the organs due to hypervitaminosis D are, in practically every case, preceded by demonstrable injury to the tissue. Calcification of the muscles of the heart and stomach is preceded by a specific type of degeneration, whereas the type of change leading to calcification of the arterial walls is less definite. In the heart, as these experiments showed, calcification is preceded by sclerosis of the coronary arteries and the degeneration of small focal areas of muscle fibers, generally located near sclerotic blood vessels.

In the kidney, the first sign of calcification is the appearance of calcareous casts formed around degenerated epithelial cells and débris in the tubules. Later the tubular epithelium opposite the localization of the casts undergoes degeneration and calcification. Degeneration of the renal parenchyma occurs in relation to sclerosis of the interlobular arteries and their branches. This is followed by calcification.

It would appear that calcium deposits occurring in hypervitaminosis D have a predilection for elastic tissue, as evidenced by the regularity of the deposits along the elastic fibers in the blood vessels and the

involvement of the elastic tissue of the walls of bronchi, as well as for tissues suffering hyaline degeneration.

The earliest changes in elastic fibers are difficult to demonstrate, so that it is possible that these fibers undergo some type of degeneration prior to the deposition of calcium along their outer surfaces.

In these experiments there was no indication that excessive doses of irradiated ergosterol cause any morphologic change in the parathyroid glands of the rabbit, either in the form of a hyperplasia or of a degenerative process in the parenchyma of the glands.

SUMMARY

The arteriosclerosis produced by hypervitaminosis D is not specific.

The earliest calcareous deposits in the blood vessels are in the form of an incrustation about the elastic fibers in the media, and are preceded by some change in the adjacent intercellular substance. Degeneration of the muscle cells of the media appears at a later period.

In these experiments, the order of involvement of tissues showing calcareous deposits as a result of hypervitaminosis D, other than arteries, was: kidneys, stomach, heart, liver and lungs.

Tissue degeneration due to a toxic process precedes calcification of these organs.

The proliferative lesions occurring in the intima in arteries are not dependent on a primary degeneration of the media.

No changes occurred in the bone marrow or in the parathyroid glands comparable to those found in hyperparathyroidism.

EARLY CELLULAR REACTION TO TUBERCLE BACILLI

A COMPARISON OF THIS REACTION IN NORMAL AND TUBERCULOUS GUINEA-PIGS AND IN GUINEA-PIGS IMMUNIZED WITH DEAD BACILLI *

ESMOND R. LONG, M.D.

ARTHUR J. VORWALD, PH.D.

AND

LILIAN DONALDSON, M.A.

CHICAGO

The character of the cellular response to infection with tubercle bacilli has been the subject of long continued active investigation. Especial emphasis has in recent years been laid on the monocyte, which Cunningham, Sabin and their co-workers [1] identified as the precursor of the epithelioid cell. Sabin, Doan and Forkner,[2] moreover, credited certain specific chemical constituents of the tubercle bacillus, obtained from its lipoid fraction, with a selective affinity for and action on this cell. The monocytes entering into the early formation of the tubercle, according to their view, come in part from monocytes of the blood stream, but to a larger extent from primitive monocytes already present among the fixed elements of the infected tissues. Other investigators have traced the origin of the tubercle to tissue cells of the histiocytic or clasmatocytic class, in general to cells of the so-called "reticulo-endothelial system," which vary in appearance and development according to the exigencies of their environment. The monophyletic school of hematologists have refused to make fundamental distinctions in the nature of the reacting mononuclear cells, tracing them all back, in spite of their great morphologic variation in individual lesions, to a single stem cell (e. g., Maximow [3]).

The intensity of interest in the mononuclear cells entering into the formation of the epithelioid tubercle has drawn attention away from the

---

* Submitted for publication, Aug. 1, 1931.

* From the Department of Pathology, University of Chicago.

1. Cunningham, R. S.; Sabin, F. R.; Sugiyama, S., and Kindwall, J. A.: Bull. Johns Hopkins Hosp. **37**:231, 1925.

2. Sabin, F. R.; Doan, C. A., and Forkner, C. E.: J. Exper. Med. (supp. 3) **52**:1, 1930.

3. Maximow, A. A.: J. Infect. Dis. **34**:549, 1924.

cell long known to be the first responding element on infection with tubercle bacilli, but not considered of much significance in tubercle formation, viz., the neutrophilic polymorphonuclear leukocyte. One of us (Dr. Vorwald;[4] see his paper for literature on the subject) recently emphasized this earliest response as of genuine importance in the development of the tubercle. He stressed the fact that investigations on the "early" formation of the tubercle, dealing with its development in the first few days, have largely overlooked the truly early changes occurring in the first few hours. In a study of tubercle formation in the rabbit's lung after intravenous infection with the human type of tubercle bacilli, he showed that an initial intense intracapillary leukocytic response occurs, which is spectacular at one hour. Even at this early period, it is difficult to find a tubercle bacillus not taken up by a polymorphonuclear leukocyte. His results indicate clearly that cells of this group in a highly effective way concentrate into small sharply localized cellular masses the bacilli introduced into the general blood stream. These masses are the groundwork on which the tubercle develops. The polymorphonuclear response under the conditions of this experiment thus definitely determines the location of development of the future tubercles. As the hours go by (the process is particularly well seen at the period from the fourteenth to the eighteenth hour), large mononuclear exudate cells gradually infiltrate the polymorphonuclear mass, phagocytose the cells of this mass, and at the same time take over into their own cytoplasm the tubercle bacilli previously ingested by the neutrophilic leukocytes. An almost complete replacement thus takes place, so that at twenty-four hours a tubercle is seen that is predominantly mononuclear, with the tubercle bacilli almost exclusively in cells of this class. Subsequent modifications of these cells, by the nuclear and cytoplasmic changes that Cunningham, Sabin and their co-workers [1] and Sabin, Doan and Forkner [2] described, convert them into typical epithelioid cells.

The results just described occur on primary infection. It is well known that important differences from the characteristics of primary infection occur when tubercle bacilli are introduced into the tissues of animals already infected with tuberculosis. It is likewise true that previous treatment with dead tubercle bacilli modifies the course of subsequent infection with virulent tubercle bacilli. Animals thus treated not only react positively to tuberculin, but possess a considerable degree of immunity.[5] Moreover, in animals immunized by treatment with dead bacteria, in addition to the general immunity, an exceptionally high local

4. Vorwald, A. J.: Am. Rev. Tuberc., to be published.

5. Petroff, S. A.: Am. Rev. Tuberc. **7**:412, 1923. Lange, B.; Freund, R., and Jochimsen, E.: Ztschr. f. Hyg. u. Infectionskr. **109**:426, 1927.

immunity has been demonstrated. This was especially well shown by Gay and his associates [6] for streptococci in the pleural cavity and by Cannon and Pacheco [7] for staphylococci in the skin. In view of all these facts it seemed desirable to supplement Vorwald's experiments on primary infection with a study of the reaction at early hours in animals the reacting capacity of which had been modified by previous infection and artificial immunization.

For a number of reasons, the testis of the guinea-pig was chosen for this experiment. In the first place, it probably exhibits the allergic reaction of reinfection with a higher degree of intensity than any other organ of the body.[8] Parenchymatous degenerative changes and interstitial inflammatory changes are both profound. Secondly, the external location and isolated character of the testis render it especially suitable for an experiment in which exact and equal dosage in a series of animals is desirable. Finally, the choice of this organ made possible a comparison of the effects of general and local immunization on the development and course of a subsequently induced local tuberculosis. For this phase of the experiment, one testis was locally immunized by several injections of dead tubercle bacilli. Animals of this group thus could be used for a comparative study of the effects of local and general immunization in the same animal, the reaction to live tubercle bacilli in the immunized testis serving as an index of the effect of local immunization, and the reaction to an equal dosage of bacilli in the other testis, as an index of the degree and character of general immunization.

PLAN OF EXPERIMENT

Three groups of guinea-pigs were used: (1) normal guinea-pigs, (2) guinea-pigs rendered tuberculous by an injection of virulent human type tubercle bacilli (Saranac Lake H_{37}) one month previously into the left axilla, and hypersensitive to tuberculin at the time of the experiment, as proved by cutaneous tuberculin tests, and (3) guinea-pigs that had been treated by five injections of 0.1 mg. of a suspension of heat-killed human type tubercle bacilli (H_{37}) at intervals of two, four, two and four days and that had been allowed to rest one month after the fifth injection of the dead bacilli. Several facts of interest developed in connection with this immunizing procedure. As was anticipated from the work of Gay and his associates [6] and that of Cannon and Pacheco,[7] extensive scarring resulted, with the production of a moderately vascular granulation tissue extensively infiltrated by cells of inflammation, especially lymphocytes and large mononuclear exudate cells. The seminiferous tubules were not wholly destroyed by the procedure, many persisting with characteristic spermatogenesis.

---

6. Gay, F. P.; Clark, A. R., and Linton, R. W.: Histologic Basis for Local Resistance and Immunity to Streptococcus: VIII. Studies in Streptococcus Infection and Immunity, Arch. Path. **1**:857, 1926.

7. Cannon, P. R., and Pacheco, G. A.: Am. J. Path. **6**:749, 1930.

8. Long, E. R.: J. Infect. Dis. **37**:368, 1925.

Infecting injections were then made as follows: 0.1 mg. of H_{37} tubercle bacilli per kilogram of animal weight (about 0.05 mg. per animal) was injected into the left testis in group 1, the left testis in group 2 and both testes in group 3. Animals of each group were then killed at the end of one, three, six, ten, sixteen, twenty-four, forty-eight and seventy-two hours. The testes into which the injections had been made were fixed in Zenker's fluid plus formaldehyde and stained with hematoxylin and eosin for general observation, eosin-azure for particular study of the leukocytes, and fuchsin, hematoxylin and orange-G for study of the tubercle bacilli.

OBSERVATIONS: AT ONE HOUR

Normal Guinea-Pig, Left Testis.—Some bacilli were found clumped outside of cells; occasionally polymorphonuclear leukocytes were in their neighborhood, and a few bacilli were within polymorphonuclears. The blood vessels contained an excess of polymorphonuclears. Tubercle bacilli were not seen in mononuclear cells. In general, little reaction was present; on superficial examination, the sections would be taken for those of a normal testis.

Tuberculous Guinea-Pig, Left Testis.—Bacilli in moderate number were clumped in groups of polymorphonuclear leukocytes and in clumps outside. Allergic reaction, as manifested by an abnormal degree of exudation and degeneration of parenchymatous cells, was not yet apparent. In general, the testis looked like the left testis of the normal guinea-pig.

Immunized Guinea-Pig, Left Testis (Locally Immunized).—The testis was small, and its tubules were atrophied; in general, it consisted of scar tissue infiltrated by cells of inflammation of all types, fibroblasts predominating (the effect of local immunization). In one area there was a region of suppuration. Tubercle bacilli were seen, occasionally extracellular, occasionally in polymorphonuclears and occasionally in large mononuclears.

Immunized Guinea-Pig, Right Testis.—Bacilli were found outside of cells, without appreciable reaction; others were in the midst of a group of polymorphonuclear leukocytes. In each case, they were clumped. The testis was otherwise unchanged.

Summary.—The reaction at this period was slight in every case. No striking difference was seen in the different groups. A tendency to clumping of the bacilli was seen in all testes. What little reaction there was was chiefly on the part of polymorphonuclear leukocytes, which had concentrated the bacilli in groups and accomplished some phagocytosis. In the locally immunized testis, where numerous large mononuclears were already present, some phagocytosis by this type of cell had occurred, but it was not conspicuous.

AT THREE HOURS

Normal Guinea-Pig, Left Testis.—Clumps of bacilli were found in masses of polymorphonuclear leukocytes, approximately 15 to 20 leukocytes per group in a single section plane. These groups were concentrated in the center of the organ.

Tuberculous Guinea-Pig, Left Testis.—The testis was hyperemic and definitely the seat of an early tuberculin reaction, with barely recognizable degeneration of germ cells, but moderately extensive, diffuse intertubular infiltration by cells of inflammation. These were chiefly polymorphonuclear leukocytes and eosinophils. Tubercle bacilli were found in polymorphonuclear leukocytes and not extracellularly.

Immunized Guinea-Pig, Left Testis (Locally Immunized).—The testis was much scarred and contained cells of inflammation of all sorts, including many eosinophils. Spermatogenesis was not entirely destroyed. Tubercle bacilli were concentrated in accumulations of polymorphonuclear leukocytes and not in the large mononuclear cells present.

Immunized Guinea-Pig, Right Testis.—Near the center of the organ was a region of accumulation of polymorphonuclear leukocytes. Among these leukocytes were many clumps of tubercle bacilli. The region of accumulation had a diameter equal to that of two tubules.

Summary.—The reacting cell was the polymorphonuclear leukocyte in all instances, including the locally immunized testis already infiltrated by large mononuclears with phagocytic capacity. The testis of the tuberculous animal, even at this early stage, showed evidence of its general hypersensitiveness in the extent and diffuseness of the intertubular infiltration and the slight degenerative changes in the germ cells.

AT SIX HOURS

Normal Guinea-Pig, Left Testis.—There was no appreciable tubular degeneration. Small accumulations of polymorphonuclear leukocytes containing tubercle bacilli were now present between the tubules. There were similar accumulations between tubules, without tubercle bacilli.

Tuberculous Guinea-Pig, Left Testis.—The tubules were swollen and the germ cells degenerated. There was an intense exudation of cells of inflammation and fibrin between the tubules. The reaction was more diffuse than in the immunized guinea-pig. The cells of the general exudate were chiefly polymorphonuclears, but a moderate number of mononuclears and a moderate number of eosinophils were also present. Here and there were small accumulations of polymorphonuclears, resembling small abscesses, in which tubercle bacilli were clumped.

Immunized Guinea-Pig, Left Testis (Locally Immunized).—The testis was much scarred and infiltrated by cells of inflammation of all sorts; eosinophilic leukocytes were especially conspicuous. At one margin of the testis, there was a large old abscess with a necrotic center surrounded by polymorphonuclears. Scattered throughout the testis were small intertubular abscesses made up of polymorphonuclear leukocytes containing tubercle bacilli. The mononuclears, which were so prominent a part of the picture (as a result of the local immunization), did not take part in the reaction to the tubercle bacilli. No phagocytosis by eosinophils was seen, although these cells were present in enormous number.

Immunized Guinea-Pig, Right Testis.—The tubules were normal, but throughout the testis there was an intertubular infiltration by cells of inflammation, among which eosinophils were extremely numerous. Small, dense accumulations of polymorphonuclear leukocytes containing tubercle bacilli were between the tubules. Eosinophils were extremely numerous in the blood vessels. The cells immediately around the bacilli were almost exclusively polymorphonuclear neutrophils. Aside from the scarring previously produced in the locally immunized testis, the reaction was the same in the right and the left testis of this guinea-pig.

Summary.—A difference in the degree and character of the reaction in the different groups was now readily apparent. In the normal guinea-pig, the reaction was slight and chiefly polymorphonuclear. In the tuberculous guinea-pig, an intense inflammatory reaction had developed, and in further contrast to the normal animal, the parenchymatous cells showed beginning degeneration (a well recognized feature of the tuberculin reaction in the testis). A feature specifically characteristic in

this animal was the exudation of fibrin. In the animal immunized by injection of dead bacilli, the intensity of the cellular response lay between that of the normal and that of the tuberculous animal. The difference from the normal indicated a distinct allergic element. Absence of tubular degeneration in the right testis of the immunized animal showed that vaccination of the animal with dead bacilli had not brought about as intense sensitization of the parenchymatous cells as actual tuberculosis would have. A noteworthy feature of the allergic reaction was the exudation of eosinophilic leukocytes, which, in contradistinction to the neutrophils, carried out no phagocytosis. The actively reacting cell in all groups was the polymorphonuclear neutrophil. This was true of the locally immunized testis, in spite of the fact that it was full of mononuclears before the injection of tubercle bacilli (fig. 1 *A* and *B*; fig. 2 *A* and *B*).

AT TEN HOURS

Normal Guinea-Pig, Left Testis.—There was no appreciable tubular degeneration. There was definite but only moderate intertubular infiltration by cells of inflammation. Most of these were polymorphonuclear leukocytes, but many plasma cells and some lymphocytes and eosinophils were also present. Eosinophils were fewer than in the immunized animals. Tubercle bacilli were found in clumps in and among polymorphonuclear leukocytes.

Tuberculous Guinea-Pig, Left Testis.—Polymorphonuclear leukocytes were extremely numerous in the small blood vessels. In some vessels, these cells were accumulated to such a degree as to produce the picture of small septic thrombi (fig. 3). No bacilli were found in these intravascular leukocytes. Otherwise, the picture resembled that at six hours.

Immunized Guinea-Pig, Left Testis (Locally Immunized).—This was a typical immunized testis, uniformly scarred with connective tissue and infiltrated by lymphocytes and large mononuclear exudate cells. Bacilli were found in polymorphonuclear leukocytes, which were present in small scattered masses, although in this instance only in small numbers.

Immunized Guinea-Pig, Right Testis.—The tubules showed very slight degeneration. On the whole, the picture resembled that at six hours, except that there was a larger accumulation of polymorphonuclear leukocytes with tubercle bacilli near the center of the organ.

Summary.—The picture differed from that at six hours chiefly in that, in general, the reactions were more intense. Parenchymatous degeneration was now becoming apparent in the right testis of the guinea-pig immunized with dead bacilli, an effect not yet noticeable in the normal animal, evidence that vaccination with dead bacilli sensitized these cells to some extent. The degree of this degeneration was much greater in the tuberculous animal than in the animal immunized with dead bacilli. The polymorphonuclear leukocytic response was still the dominant reaction, and its intensity in the tuberculous and immunized animals greatly surpassed its intensity in the normal.

AT SIXTEEN HOURS

Normal Guinea-Pig, Left Testis.—Tubular degeneration was hardly appreciable, but there was a great increase in intertubular infiltration by cells of inflammation, and in these intertubular infiltrations were small definite abscesses consisting of polymorphonuclear leukocytes with tubercle bacilli.

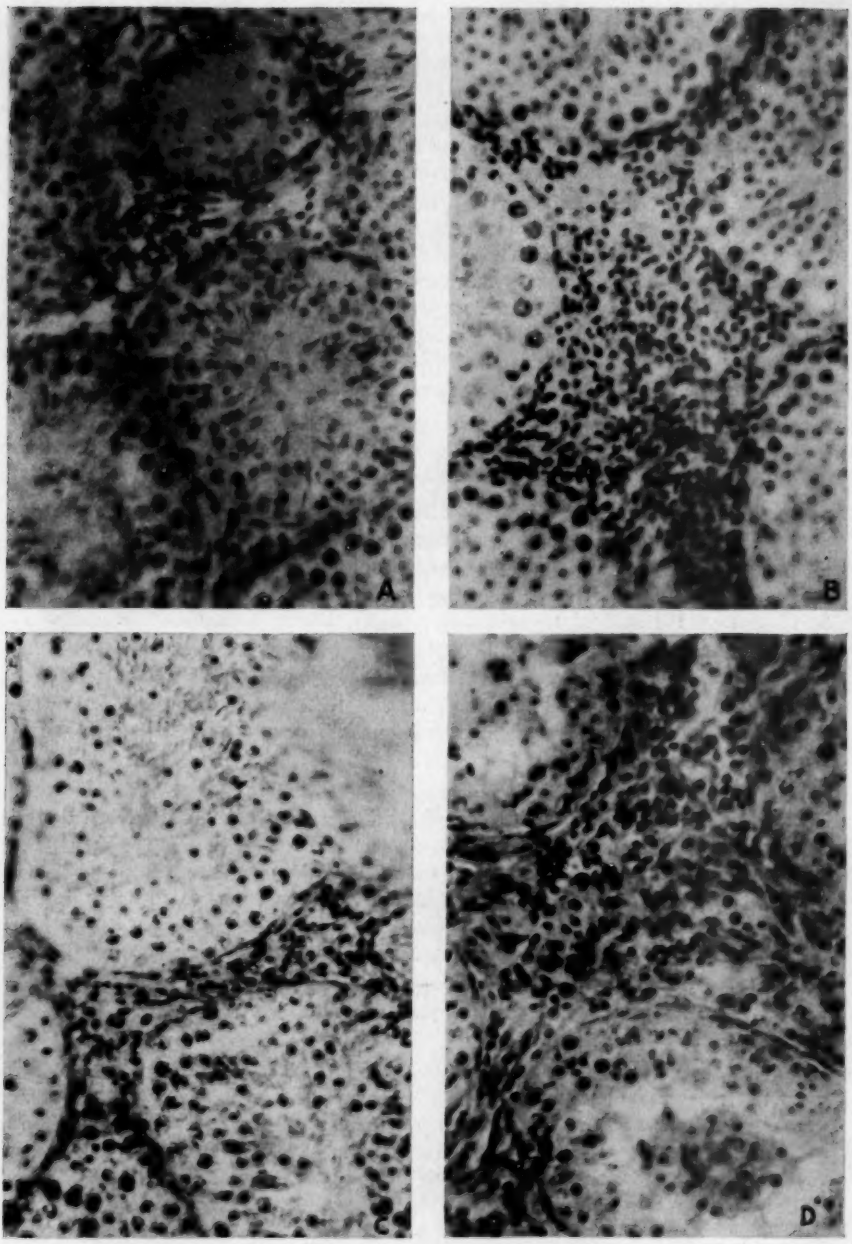


Fig. 1.—*A*, the left testis of a normal guinea-pig six hours after an injection of virulent tubercle bacilli. Note the margination of leukocytes in the blood vessel and the slight exudate between the seminiferous tubules. *B*, the left testis of a tuberculous guinea-pig six hours after an injection of virulent tubercle bacilli. Note the large size of the intertubular exudate in comparison with that in *A*. *C*, the left testis of a normal guinea-pig seventy-two hours after an injection of virulent tubercle bacilli. Note that the intertubular exudate now consists chiefly of mononuclear cells. *D*, the left testis of a tuberculous guinea-pig seventy-two hours after an injection of virulent tubercle bacilli. Note that the intertubular exudate is much larger than in *C* and approximately equal in size to that in *B*; it now consists chiefly of mononuclear cells. Note the degeneration of the seminiferous tubules.

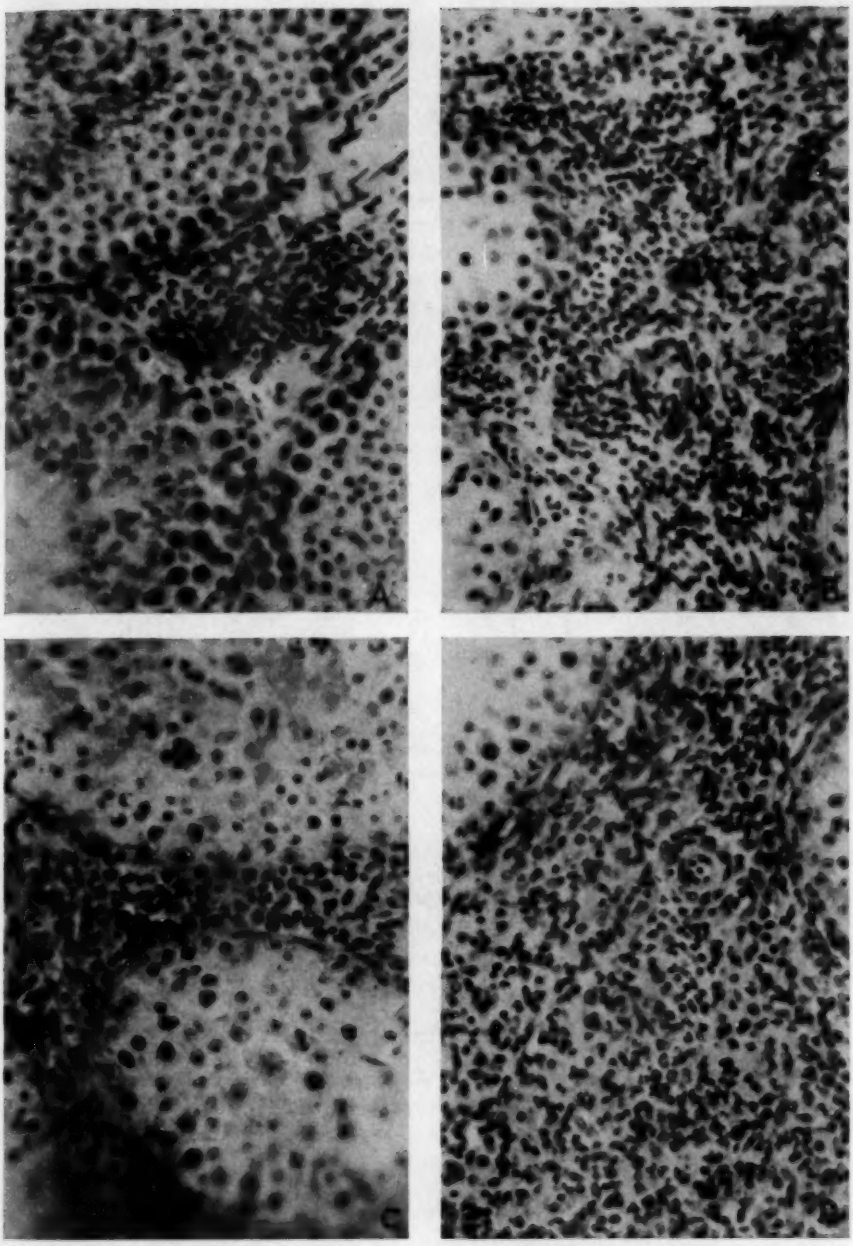


Fig. 2.—*A*, the right testis of a guinea-pig immunized by repeated injections of dead tubercle bacilli into the left testis, six hours after an injection of virulent tubercle bacilli. Note the exudation of polymorphonuclear leukocytes between tubules. The size of the region of exudation is between that seen in figure 1 *A* and *B*. *B*, the left testis from the same animal as *A*, also six hours after an injection of virulent tubercle bacilli. Note the extensive mononuclear leukocyte infiltration and the tubular degeneration due to local immunization with dead bacilli. Note also the extensive infiltration by polymorphonuclear leukocytes due to the recent injection of live tubercle bacilli. *C*, the right testis of a guinea-pig immunized by repeated injections of dead tubercle bacilli into the left testis, seventy-two hours after an injection of virulent tubercle bacilli. Compare with *A*, and note that the exudate now consists chiefly of mononuclear leukocytes. *D*, the left testis from the same animal as *C*, also seventy-two hours after an injection of virulent tubercle bacilli. The intertubular exudate consists almost entirely of mononuclear cells, the polymorphonuclear leukocytes characteristic of the reaction in *B* having been replaced.

Tuberculous Guinea-Pig, Left Testis.—The reaction was a typical one of reinfection, but happened in this case to be less marked than at ten hours. Tubercle bacilli were seen in polymorphonuclear leukocytic abscesses as usual. Large mononuclear leukocytes were beginning to mingle with polymorphonuclears in these abscesses.

Immunized Guinea-Pig, Left Testis (Locally Immunized).—The reaction was essentially the same as previously described for the comparable testis in the ten hour animal.

Immunized Guinea-Pig, Right Testis.—Tubular degeneration was now definite, but less intense than in the tuberculous animal. Intertubular infiltration was increasing greatly, with many dense accumulations of polymorphonuclears containing tubercle bacilli.

Summary.—The picture was much like that at ten hours.

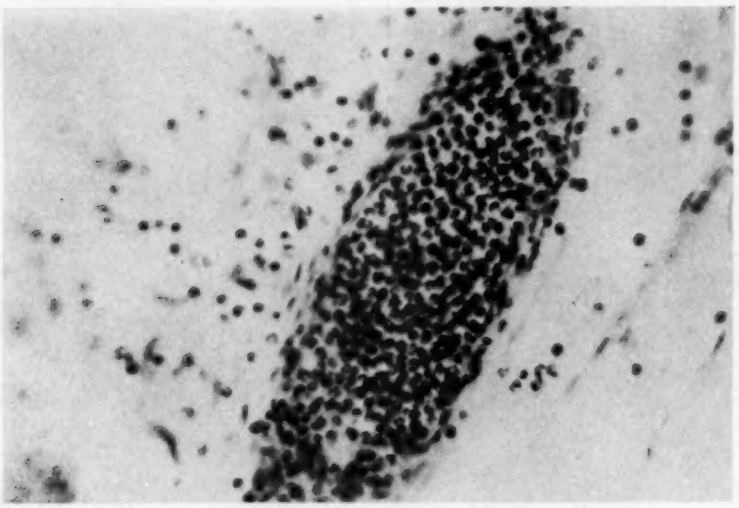


Fig. 3.—A blood vessel in a zone of inflammatory edema in the testis of a tuberculous guinea-pig, packed with polymorphonuclear leukocytes ten hours after an injection of virulent tubercle bacilli. This powerful chemotaxis for leukocytes is one manifestation of a tuberculin reaction.

AT TWENTY-FOUR HOURS

Normal Guinea-Pig, Left Testis.—Tubular degeneration was now beginning to be noticeable. Intertubular infiltration by cells of inflammation was like that at sixteen hours, but more concentrated in spots. Tubercle bacilli were present in the accumulations of polymorphonuclear leukocytes.

Tuberculous Guinea-Pig, Left Testis.—A tuberculin reaction of profound severity was present, with intense degeneration of the tubular cells and exudation of cells of inflammation and fibrin. Tubercle bacilli were still found, for the most part, in polymorphonuclears; nevertheless, large mononuclears were beginning to appear in these regions, among the polymorphonuclear leukocytes.

Immunized Guinea-Pig, Left Testis (Locally Immunized).—Less scarring than usual had resulted from the local immunization. Small polymorphonuclear abscesses with bacilli in and among the leukocytes were still found in the regions of lymphocytic and mononuclear infiltration due to the immunizing.

Immunized Guinea-Pig, Right Testis.—Tubular degeneration was apparent, but not marked. Otherwise, the picture was the same as in the corresponding testis at sixteen hours.

Summary.—The reaction of hypersensitiveness in the testis of the tuberculous guinea-pig was now at its height; its chief features were intense intertubular exudation, in which fibrin and leukocytes were abundant, and profound tubular degeneration. The right testis of the animal immunized by injections of dead bacilli into the left testis was the seat of a moderately diffuse intertubular exudate in which fibrin was less conspicuous; tubular degeneration had occurred, but was only moderate. In the left testis, locally immunized, the reaction to the recently introduced bacilli was still polymorphonuclear leukocytic. In the previously normal guinea-pig the reaction was less intense than in any of the others, and was less diffuse; twenty-one hours later than in the tuberculous guinea-pig parenchymatous degeneration was beginning to be seen.

AT FORTY-EIGHT HOURS

Normal Guinea-Pig, Left Testis.—Tubular degeneration was now definite, although slight as compared with that in a tuberculin reaction. Intertubular cellular infiltration had increased, but the cells were now predominantly large mononuclears. Definite concentrations of polymorphonuclear leukocytes, equivalent to abscesses, were present and full of tubercle bacilli. The number of bacilli in these accumulations was extraordinary; the colony-like arrangement, as well as the number of bacilli present, was such as to suggest actual bacillary multiplication. The bacilli were unusually granular.

Tuberculous Guinea-Pig, Left Testis.—This testis had a tubercle in it (hematogenous from the original infection). Outside of this, it showed a profound tuberculin reaction. Regions of polymorphonuclear accumulation and others made up chiefly of mononuclears were present.

Tuberculous Guinea-Pig, Right Testis (An injection was made into the right testis because of the condition of the left).—There was a typical tuberculin reaction, though tubular damage was not so profound. The cells reacting to the tubercle bacilli were now about 50 per cent polymorphonuclears and 50 per cent large mononuclears. A conspicuous feature was the phagocytosis by large mononuclear leukocytes of polymorphonuclears containing tubercle bacilli.

Immunized Guinea-Pig, Left Testis (Locally Immunized).—The testis was much scarred, with large areas of lymphocytic infiltration (from the immunizing procedure). The lymphatics in this region were packed with lymphocytes (local production of lymphocytes). Throughout the region of lymphocytes were accumulations of polymorphonuclears, virtually small abscesses in the midst of the regions of lymphocytic infiltration; in them, tubercle bacilli were numerous. Most of the bacilli were in polymorphonuclears, but some were in large mononuclears. The mononuclears now made up about 25 per cent of the cellular infiltration. The polymorphonuclear leukocytes were disintegrating, and there was a good deal of phagocytosis of débris, tubercle bacilli and polymorphonuclears by the large mononuclears.

Immunized Guinea-Pig, Right Testis.—Tubular degeneration was conspicuous and intertubular infiltration greatly increased. From 50 to 75 per cent of the reacting cells were polymorphonuclear leukocytes and eosinophils; 25 per cent or more were mononuclears. The bacilli were now found especially in the large mononuclears.

Summary.—The chief change that had occurred was the infiltration by large mononuclear leukocytes of the foci of polymorphonuclear leukocyte concentration. The mononuclears were actively phagocytosing the polymorphonuclears with their bacillary content. The mononuclear reaction was of approximately equal proportionate intensity in all groups of animals. It was no more conspicuous in the locally immunized testis than in the others. The intensity of total reaction in the several groups varied in the same manner as at previous hours.

AT SEVENTY-TWO HOURS

Normal Guinea-Pig, Left Testis.—Tubular damage was apparent, although still very slight as compared with that in a tuberculin reaction. The regions of cellular infiltration had distinctly decreased in size; they were now scattered and compact and the cells present were chiefly mononuclears, and there was much phagocytosis of bacilli by mononuclears; phagocytosis was now practically confined to this type of cell.

Tuberculous Guinea-Pig, Left Testis.—The picture was that of a typical advanced tuberculin reaction. There was extreme degeneration of the tubules. Interstitial infiltration by cells of inflammation was extensive; these were now in a large majority mononuclears. Much fibrin was still present. Polymorphonuclears were few; they were seen occasionally phagocytosed by mononuclears. Mitotic figures in the exudate cells were occasionally seen. Bacilli were hard to find, but when present were seen in large mononuclear cells.

Immunized Guinea-Pig, Left Testis (Locally Immunized).—The typical picture of immunization scarring was present. Polymorphonuclear abscesses had practically disappeared. Remains of such abscesses could be distinguished, heavily infiltrated with large mononuclears. Polymorphonuclears were few. Tubercle bacilli were in the mononuclears, and the bacilli were smaller and more faintly staining.

Immunized Guinea-Pig, Right Testis.—The picture was that of a tuberculin reaction. There was marked degeneration of tubules, with intense interstitial infiltration; the cells of the latter, however, were almost exclusively mononuclears. The infiltration, while diffuse throughout the testis, was much more compact than before; where formerly it had been a collection of cells, now it began to resemble a tissue. Polymorphonuclears were very few. Bacilli were found in the mononuclears. Phagocytosis of polymorphonuclears containing bacilli by mononuclears could be seen. These bacilli were small, thin and pale staining.

Summary.—Throughout the series, the polymorphonuclear leukocytic response, which was so prominent at periods up to twenty-four hours, had practically disappeared. In all cases, large mononuclear exudate cells had invaded the leukocytic accumulations, phagocytosing the polymorphonuclears and taking over their content of bacilli. This change had brought about a general reduction in the size of the reacting zones. This was accompanied by a developing compactness, which gave to the zones of inflammatory reaction the character of a tissue, rather than of a collection of exuded cells. Degeneration of tubular epithelium was apparent in all cases, most intense in the tuberculous animal, least intense in the normal and of medium intensity in the animal immunized by previous injection of dead bacilli (fig. 1, *C* and *D*; fig. 2, *C* and *D*).

Figures 1 and 2 illustrate the difference in reaction in the four groups at six and seventy-two hours. In figure 1, the reactions in the normal and tuberculous animals at six and seventy-two hours are compared. In figure 2, the same comparison is made for the locally immunized testis and the opposite testis of the same animal.

COMMENT

A distinct positive correlation is apparent between the intensity of inflammatory reaction in these experiments and the presumable degree of immunity of the animals. Present-day researches indicate that guinea-pigs immunized with dead tubercle bacilli resist invasion of live bacilli better than normal animals, while animals with mild actual tuberculosis resist new infection still more strongly than animals immunized with dead bacilli. The range of immunity to fresh tuberculous infection is thus as follows: least in normal guinea-pigs; intermediate in guinea-pigs immunized with dead bacilli; greatest in guinea-pigs with mild active tuberculosis. In the experiments here recorded, the intensity of inflammatory reaction to freshly introduced tubercle bacilli varied in the same order; that is, it was least in normal animals, of intermediate grade in guinea-pigs immunized with dead bacilli and greatest in those with already present tuberculosis.

The correlation is so striking as to appear more than a coincidence, and it would be easy to assume that the more resistant state of the immunized and tuberculous animals was directly due to the intensity of cellular reaction brought to bear on the freshly introduced bacilli. Certainly this reaction brought about a localization of bacilli in small discrete foci in all animals studied. Nevertheless, as studies by Vorwald (in press) indicate, intensity of inflammatory reaction in tuberculosis cannot always be correlated directly with resistance. In a series of animals of greatly varying native immunity, such a correlation did not exist. Therefore, the results here recorded are published simply to furnish a quantitative comparison of the early inflammatory reaction at successive time periods in the four states of reactive capacity studied. Certain other factors are still to be evaluated before the exact relation of this variation in reaction intensity to actual immunity is known.

SUMMARY

Virulent tubercle bacilli (Saranac Lake strain H_{37}), in a dosage of 0.1 mg. per kilogram of animal weight, were injected into the testes of normal and tuberculous guinea-pigs and guinea-pigs previously treated by repeated intratesticular inoculation of a suspension of dead tubercle bacilli. The last named group of animals served a double purpose, in that inoculation of live bacilli into the testis into which dead bacilli had been previously injected elicited a response in a tissue that might be considered locally immunized, while injection in the opposite nontreated

testis elicited a response presumably characteristic of a general immunized state of the animal. The testis was chosen because the reactions of hypersensitiveness in this organ in the guinea-pig are intense.

The cellular reactions in these four states of testis tissue (normal testis of the normal animal, normal-appearing testis of the tuberculous animal, scarred testis of the locally immunized animal and the normal-appearing opposite testis of the same animal) were examined one, three, six, ten, sixteen, twenty-four, forty-eight and seventy-two hours after the inoculation of live virulent bacilli. In all cases, the most actively reacting cell at the early hours was the polymorphonuclear leukocyte. Phagocytosis of bacilli by these cells was practically complete in three hours. The most intense exudation of these cells at the early periods occurred in the testes of the tuberculous animals. In this group, a great deal of fibrin appeared in the exudate, and at the same time a rapid degeneration of the seminiferous tubules occurred. (This combination of changes is characteristic of the tuberculin reaction in the testis, and is due, as previous studies have shown, to the protein of the tubercle bacillus.) The next most intense exudation of polymorphonuclear leukocytes occurred in the guinea-pigs treated with repeated injections of dead tubercle bacilli. The reaction was of approximately equal intensity in the locally immunized testis and the opposite testis modified in reactive capacity only by the general immunization conferred by the local immunizing procedure. Special attention is called to the condition of the locally immunized testis. Although this testis was full of mononuclear cells of inflammation as a result of its repeated inoculation with dead bacilli, the early reaction to freshly introduced live bacilli was a polymorphonuclear one, just as in all other animals. The least intense exudative reaction occurred in the testes of the normal animals.

Between twenty-four and forty-eight hours after the inoculation with live bacilli, a gradual replacement of the polymorphonuclear leukocytes by large mononuclear exudate cells occurred in all animals. Extensive phagocytosis of polymorphonuclear leukocytes by large mononuclears took place. In this way, a transference of tubercle bacilli from the cytoplasm of the polymorphonuclears to that of the large mononuclears was accomplished. This rôle of the large mononuclear leukocytes did not appear earlier in the locally immunized testes than in the other testes of the series. At seventy-two hours, the familiar picture of scattered early mononuclear cell tubercles was present, each of these tubercles representing, it should be noted, a previous site of focalization of polymorphonuclear leukocytes and bacilli. Maturation of these large mononuclears to the typical epithelioid appearance had not yet taken place. (Other studies have shown that, on a large scale, this takes place in about two weeks.)

CONCLUSIONS

It may be concluded from these experiments that prompt localization of tubercle bacilli is brought about by exudation of polymorphonuclear leukocytes and phagocytosis of the bacilli by these cells. Although the bacilli are at first widely spread and the initial polymorphonuclear reaction is correspondingly diffuse, aggregation of the leukocytes, containing all the bacilli injected, into distinct and separate foci, soon occurs. The site of future true tubercle formation is thus determined, for the polymorphonuclears are soon replaced by large mononuclears, which, as shown by the experience of many other investigators, later develop into epithelioid cells with more abundant cytoplasm. The fact that replacement of the polymorphonuclear mass by large mononuclears did not occur appreciably earlier in the locally immunized testis, already full of mononuclear cells, than in the other testes of the series suggests that in these experiments, at least, the large mononuclear cells concerned in the reaction came directly from the blood stream and were not derived locally. An already present tuberculosis in the animal greatly accelerates the reacting capacity of the polymorphonuclear leukocytes. This may be looked on as simply one feature of a typical tuberculin reaction. Previous inoculation with dead bacilli brings about a similar allergic state, although the acceleration and increased intensity of inflammatory reaction over that seen in the normal animal are not nearly so great as in the truly tuberculous animal.

General Reviews

PELLAGRA

ETIOLOGY (MODERN THEORIES) AND PATHOLOGIC ANATOMY *

HARVEY S. THATCHER, M.D.
LITTLE ROCK, ARK.

Pellagra has been related to warm climates since its discovery in Spain by Casal [1] in 1735. The description of the disease in France during the first part of the last century by Hameau [2] places it in the southwestern part of that country. The prevalence of pellagra in Italy early in the history of the disease, reported in the important monographs of Frapolli,[3] Zanetti,[4] Strambio,[5] Gherardini [6] and others, associated the disease with climatic conditions. The invasion of the United States by pellagra during the past two decades has been confined, as is generally known, mainly to the southern states.

Maize was observed to be the principal article in the food of pellagrins in the lower latitudes. The maize theory of the etiology of pellagra was advanced for many years, from which was evolved the deficiency theory. The relation of sunlight to the disease brought forth the photodynamic theory. The investigations of micro-organisms as a cause of other infections naturally introduced the parasitic theory. Research has been directed mainly from these hypotheses during the last twenty years.

THE DEFICIENCY THEORY

The importance of maize in the diet gave rise to the zeists, whose ideas flourished for many decades. During the last century, the maize theory was advanced by such prominent workers as Marzari,[7] Balardini,[8]

* Submitted for publication, March 28, 1931.

* From the Department of Pathology, University of Arkansas, School of Medicine.

1. Casal, D. C.: Ses memorias de historia natural y medica de Asturias, 1762; cited by Sambon: Presse méd. **24**:577, 1916.

2. Hameau, J.: Mémoire sur une maladie de la peau (peu connue) observeé dans les environs de la teste; cited by Roussel: La pellagra, Paris, 1845.

3. Frapolli, F.: Animadversinoes in morbum vulga pelagram (sic) Milan, 1771; cited by Roussel.

4. Zanetti, cited by Roussel.

5. Strambio, G., Sr., cited by Roussel.

6. Gherardini: Geschichte des Pellagra; cited by Roussel.

7. Marzari: Saggio medico politico; cited by Roussel.

8. Balardini: Della Pellagra, Milano, 1845; cited by Harris: Pellagra, New York, The Macmillan Company, 1919.

Morelli [9] and Roussel.[10] This theory was regarded as defective when it became known that pellagra exists in countries in which Indian corn is not consumed. The extensive investigations of the Illinois commission [11] struck another blow to zeism, because it could not relate maize as an etiologic factor.

During the present century extensive search has been made to discover the exact factor that is missing in the diet. Outbreaks of the disease have occurred in which faulty diets were proved. Studies were made of the diets of pellagrins among Armenian refugees [12] and Turkish and German prisoners [13] at the time of the World War, but the exact deficiency remained somewhat uncertain. Enright,[14] after a critical analysis of the diets of German prisoners, did not believe that a food deficiency was related to this disease. MacNeal [15] noted that no outbreaks had occurred in France, England, Germany or Austria during the war. I saw hundreds of impoverished French refugees with no signs of pellagra. At that time dietary studies on protein food values were considered, and a theory developed that pellagra was caused by a deficiency of protein in the diet. Goldberger,[16] Voegtlin,[17] Goldberger, Waring and Willets [18] and Wheeler [19] gave additional proofs of the prevention or of the cure of the disease. Jobling and Peterson [20] in their extensive studies on the epidemic in Nashville noted two factors that were contradictory: A certain number of cases occurred when the diet was wholesome; at least half of the cases developed where there was a monotonous diet low in protein and high in carbohydrates. Although the protein deficiency theory was advanced, it was not established.

The late pellagrologist, Joseph Goldberger,[21] emphasized with considerable force his view that a faulty supply of protein (amino-acid)

---

9. Morelli: La pellagra, Firenze, 1855; cited by Harris.

10. Roussel: La pellagra, Paris, 1845.

11. Report of the Pellagra Commission of the State of Illinois, Springfield, Illinois State Printer, 1911; condensed report in Arch. Int. Med. **10**:123 and 219, 1912.

12. White, R. G.: Reports and Notes of the Public Health Laboratory, Cairo, Egypt, Government Press, 1919.

13. J. Roy. Army M. Corps **33**:426 and 508, 1919; **34**:70, 173 and 272, 1920.

14. Enright, J. I.: Lancet **1**:998, 1920.

15. MacNeal, W. J.: South. M. J. **15**:899, 1922.

16. Goldberger, J.: Pub. Health Rep. **29**:2354, 1914.

17. Voegtlin, C.: Pub. Health Rep. **35**:1435, 1920.

18. Goldberger, J.; Waring, C. H., and Willets, D. G.: Pub. Health Rep. **29**:2821, 1914.

19. Wheeler, G. A.: Pub. Health Rep. **39**:2197, 1924.

20. Jobling, J. W., and Peterson, W.: J. Infect. Dis. **18**:501, 1916; **21**:109, 1917.

21. Goldberger, J.: J. A. M. A. **66**:471, 1916; **78**:1676, 1922; **80**:1866, 1923. Goldberger, J., and others: Pub. Health Rep. **41**:297, 1926.

is the primary cause of pellagra. Prior to Goldberger's extensive publications the Illinois commission [11] noted that pellagra had decreased in certain hospitals with an increase in the consumption of meat. Goldberger's work was conscientiously planned and performed, but his conclusions might be questioned. The dermatitis that he produced in six convicts on a diet deficient in protein has been questioned. Lesions of the skin appeared first on the scrotum in six convicts and on the backs of the hands in two. This dermatitis has not been accepted as diagnostic of pellagra by all of the critics. MacNeal [15] and Hindhede [22] were probably correct when they considered Goldberger's [21] conclusions somewhat uncertain. The most important criticism that can be offered to this experiment on the prison farm, one which was mentioned by Jobling and Peterson,[20] is that it was not accomplished in a nonpellagrous community, even if it were demonstrated that the convicts develop unquestionable pellagrous symptoms. The experimental lesions produced in monkeys by Chick and Hume [23] on a diet low in protein could not be called pellagrous without question.

Funk [24] advanced the idea that pellagra might be caused by lack of a vitamin. Vedder [25] believed that there was resemblance between pellagra, scurvy and beriberi and compared the changes in the nervous system to those in beriberi. Since the discovery that vitamin B complex is composed of two factors, deficiency of vitamin G [26] has been considered to be the cause of pellagra. There was lack of vitamin G in the diet of pellagrins whom I [27] observed in an outbreak after the inundation in Arkansas in 1927. Irby,[28] who studied the diets of pellagrins in Arkansas after the drought, found a deficiency of vitamin G. Goldberger [29] reported the presence of vitamin G in various foods especially in yeast and in lean meat. Aykroyd and Roscoe [30] found that dried ox liver, yeast and fresh whole milk were excellent sources of vitamin B2. Since meat, milk and especially yeast were important articles required for prevention or cure, it was natural to relate vitamin G as the necessary pellagra-preventing factor. Vitamin G caused pellagra-like symptoms in the albino rat to disappear, and this vitamin

22. Hindhede, M.: J. A. M. A. **80**:1685, 1923.

23. Chick, H., and Hume, M.: Biochem. J. **14**:135, 1920.

24. Funk, C.: J. Physiol. **47**:389, 1913. Eddy, W. H.: Vitamin Manual, Baltimore, Williams & Wilkins Company, 1921.

25. Vedder, E. B.: Arch. Int. Med. **18**:137, 1916.

26. The letter "G" has been adopted by the Committee on Nomenclature appointed by the American Society of Biological Chemists to indicate the stable factor associated with the vitamin B complex. The British biochemists have provisionally adopted the term "B2."

27. Thatcher, H. S.: J. Arkansas M. Soc. **24**:193, 1928.

28. Irby, Patricia: Personal communication to the author, 1930.

29. Goldberger, J.: Pub. Health Rep. **42**:2193, 1927.

30. Aykroyd, W. R., and Roscoe, M. H.: Biochem. J. **23**:483, 1929.

was then designated as the antipellagric vitamin. Goldberger now changed his opinion to comply with the vitamin G deficiency theory.

Experimental production of pellagra-like symptoms in the albino rat has been reported by Goldberger and Lillie,[31] Chick and Roscoe,[32] Hunt,[33] Salmon, Hays and Guerrant,[34] Sherman and Sandels,[35] Findlay,[36] and Thatcher, Sure and Walker.[37] The last mentioned workers completed a critical study with histologic examinations, but could not relate the disease absolutely to human pellagra, although symmetry of the lesions and seasonal variation were observed. Recently it was suggested by Sure,[38] Salmon[39] and Hunt and Wilder[40] that vitamin G consists of two factors. Sure and Smith[41] concluded that the growth-producing factors associated with the antipellagric vitamin are not identical. Further work should give us more evidence about this so-called antipellagric vitamin.

The experimental "black tongue" syndrome of dogs was related to pellagra and lack of protein in the diet by Underhill and Mendel. Goldberger and Wheeler[42] later prevented and cured "black tongue" by autoclaved yeast. Denton[43] stated that the distinctive lesions of pellagra and those of "black tongue" of dogs appeared to have their origin in a failure on the part of the organism to maintain the specialized supporting tissues of epithelium in various situations.

Recently it was suggested that elements other than vitamins may have some significance. Leader[44] claimed that in the absence of cane sugar in the diet, pellagra did not occur in experimental rats with a diet deficient in vitamin "B complex." She noted the seasonal variation that was observed by Thatcher, Sure and Walker[45] on another diet. Bliss[46] suggested that pellagra was a nondeficiency disease. He stated

31. Goldberger, J., and Lillie, R. D.: Pub. Health Rep. **41**:1025, 1926.
32. Chick, H., and Roscoe, M. H.: Biochem. J. **21**:698, 1927.
33. Hunt, C. H.: J. Biol. Chem. **78**:83, 1928.
34. Salmon, W. D.; Hays, I. M., and Guerrant, N. B.: J. Infect. Dis. **43**:426, 1928.
35. Sherman, H. C., and Sandels, M. R.: Proc. Soc. Exper. Biol. & Med. **26**:536, 1929.
36. Findlay, G. M.: J. Path. & Bact. **31**:353, 1928.
37. Thatcher, H. S.; Sure, B., and Walker, D. J.: South. M. J. **23**:143, 1930; Arch. Path. **11**:425, 1931.
38. Sure, B.: Personal communication to the author, 1931.
39. Salmon, W. D.: J. Chem. Educ. **7**:2336, 1930.
40. Hunt, C. H., and Wilder, W.: J. Biol. Chem. **90**:279, 1931.
41. Sure, B., and Smith, M. E.: Proc. Soc. Exper. Biol. & Med. **28**:442, 1931.
42. Goldberger, J.; Wheeler, G. A.; Lillie, R. D., and Rogers, L. M.: Pub. Health Rep. **43**:1385, 1928.
43. Denton, J.: Am. J. Path. **4**:341, 1928.
44. Leader, V. R.: Biochem. J. **24**:1172, 1930.
45. Thatcher, Sure and Walker (footnote 37, second reference).
46. Bliss, S.: Science **72**:577, 1930.

that iron was present in foods containing much vitamin G, while the usual diet of the poor farmers in the South lacked iron. He further observed that dogs fed the Chittenden-Underhill diet were restored to normal by the intravenous injection of iron. Sure, Kik and Smith,[47] however, found anemia in rats suffering from vitamin G deficiency accompanied by lesions of the skin comparable to those found in human pellagra, when the diet contained plenty of ferric citrate. More evidence must be produced before the views of Leader [44] and Bliss [46] can be accepted.

From the analysis of the most important research relating to this theory, it is not clear that a deficient diet is the primary cause of pellagra, although there is a dietary relationship.

THE PHOTODYNAMIC THEORY

Fagopyrism has been regarded as of considerable importance because the dermatitis of pellagra is initiated and accentuated by sunlight. Maize was used as a source for the photodynamic substances by Hausmann,[48] Lode,[49] Horbaczewski,[50] Umnus [51] and Raubitschek.[52] Lesions were produced in animals inoculated with these substances when they were exposed to the sun's rays. Jobling and Arnold [53] did not consider it necessary to use maize as the source of the photodynamic substances because evidence had accumulated against the maize theory. They isolated organisms from the stools of pellagrins producing fluorescent substances. Extracts of these organisms were inoculated into a series of mice. When these mice were exposed to light, edema and reddening of the ears occurred, with swelling and edema of the eyelids; also gangrene of the ears developed, if inoculation and exposure were continued daily. A fungus having photodynamic properties was found in the stools of the pellagrins, which belonged to the *Aspergillus glaucus-repens* group.

Scott, Turner and Mayerson [54] found no difference between normal and pellagrous serum (acute cases) to warrant the conclusion that they were not spectroscopically identical. Comparison between normal serum containing traces of hematoporphyrin and pellagrous serum revealed no trace of it in the circulating blood of pellagrins. However,

47. Sure, B.; Kik, M. C., and Smith, M. E.: Proc. Soc. Exper. Biol. & Med. **28**:498, 1931.

48. Hausmann, W.: Wien. klin. Wchnschr. **23**:1287, 1910.

49. Lode, Sitzung: Wien. klin. Wchnschr. **23**:1160, 1910.

50. Horbaczewski, J.: Centralbl. f. Bakteriol. **58**:317, 1911.

51. Umnus, O.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **13**:461, 1912.

52. Raubitschek, H.: Wien. klin. Wchnschr. **23**:963, 1910.

53. Jobling, J., and Arnold, L.: J. A. M. A. **80**:365, 1923.

54. Scott, L. C.; Turner, R. H., and Mayerson, H. S.: Proc. Soc. Exper. Biol. & Med. **27**:27, 1929.

Meyer-Betz [55] sensitized himself to hematoporphyrin and got edema and erythema of the face, neck and hand when exposed to the sunlight. He remained hypersensitive for six weeks, although a test for hematoporphyrin showed none present after seventy-two hours. The negative results of Scott, Turner and Mayerson [54] concerning hematoporphyrin may have no significance.

Cluver [56] combined the deficiency and the photodynamic theories when he found the diets of two groups of prisoners deficient in the pellagra-preventing factor. In those who were exposed to sunlight pellagra developed, while in those who were in the shade no symptoms appeared. Jobling and Arnold [53] produced evidence that the photodynamic theory and the parasitic theory could go hand in hand.

Sellards,[57] who studied photodynamic properties of sunlight in the tropics, believed that more investigations were necessary to correlate these properties with disease. He considered that photodynamic action is associated closely with fluorescence, but that they are not invariably associated.

At present it is evident that photodynamics in relation to pellagra is comparatively ill understood.

THE PARASITIC THEORY

The parasitic theory was given impetus by Sambon [58] after careful epidemiologic studies. Prior to that time organisms had been isolated from maize, but they were not satisfactorily correlated with pellagra. Sambon [58] noticed that pellagra did not attack indiscriminately all those who lived on maize; it had marked seasonal incidence; it was not transmitted by lactation, and conjugal pellagra had been reported. He suggested as a working hypothesis that pellagra was analogous to some of the protozoan diseases. Later Sambon found that men in the Venice arsenal did not have the malady, while the fishermen on other islands were pellagrous. He associated this observation with a biting fly and chose *Simulium* as a working hypothesis. Sambon's suggestions were not substantiated by the Illinois commission [11] and others. The important epidemiologic studies of Jobling and Peterson [20] correlated the disease with unscreened houses and with access of flies to human excreta and to human habitations. A decrease of pellagra was found with an improvement in the diet.

Numerous insects were investigated by Jennings and King,[59] but no positive results were obtained. The stable fly (*Stomoxys calcitrans*)

55. Meyer-Betz, F.: Deutsches Arch. f. klin. Med. **112**:476, 1913.
56. Cluver, E. H.: Brit. M. J. **2**:751, 1929.
57. Sellards, A.: J. M. Research **38**:293, 1918.
58. Sambon, L.: Brit. M. J. **2**:1272, 1905.
59. Jennings, A. H., and King, W. V.: Am. J. M. Sc. **146**:411, 1913.

was considered by them as the possible transmitter, but the Thompson-McFadden commission [60] was unsuccessful in finding the disease in monkeys bitten by these flies previously exposed to pellagrins. The streptobacillus of Tizzoni and de Angelis [61] found on maize and in the blood, organs and cerebrospinal fluid of pellagrous persons was not found by the Illinois commission,[11] Harris [62] and others. Salmon, Hays and Guerrant [34] isolated an organism from the cutaneous lesions, parenchymatous organs, intestinal submucosa and the cavities of the involved joints of their experimental animals. They fed the organism to rats and produced lesions from which the characteristic coccus was recovered. Susman [63] claimed to have found an anaerobe in the blood of a pellagrin. The fungus found in the stools of pellagrins by Jobling and Arnold [53] has been mentioned. All of this work should be repeated with more extensive observations, so that secondary invaders may be completely eliminated.

Feeding of pellagrous tissues and fluids to monkeys and inoculation of these animals with this material were unsuccessful in producing the disease, according to the Illinois commission [11] and Francis.[64] An important contribution was made by Harris [65] which has never been corroborated. Tissues from various parts of the bodies of pellagrins were mixed with equal amounts of normal salt solution. The coarsely filtered juice was passed through a Berkefeld filter and injected subcutaneously, intravenously and intracranially in large quantities into monkeys (*Macacus rhesus*). The three animals inoculated developed all of the principal clinical signs and symptoms and pathologic evidences found in pellagra. He concluded that the cause of the disease was a filtrable virus or a micro-organism that passed through a Berkefeld filter. Francis,[64] who experimented with ninety monkeys (*M. rhesus*), three baboons and one Java monkey, could not obtain similar results. Nevertheless, Harris produced the experimental pellagra that can be least questioned.

It has been noted that pellagra is associated with other diseases. The older writers found that tuberculosis was often present. Yang and Hu [66] and Turner [67] reported the occurrence of intestinal disease in

60. Report of the Thompson-McFadden Pellagra Commission, New York, 1915.

61. Tizzoni, G., and de Angelis, G.: Centralbl. f. Bakteriol. **74**:219, 1914. Tizzoni, G.: Nuove recerche batteriologische sulla pellagra, Rendic. r. Accad. d. sc. d. Ist. di Bologna **12**:19, 1907-1908; cited by Marie (footnote 69).

62. Harris, H. F.: Pellagra, New York, The Macmillan Company, 1919.

63. Susman, W.: Edinburgh M. J. **34**:419, 1927.

64. Francis, E.: Hygienic Laboratory Bulletin 106, 1917, p. 81.

65. Harris, W. H.: J. A. M. A. **60**:1948, 1913.

66. Yang, C. S., and Hu, C. K.: Nat. M. J., China **16**:625, 1930.

67. Turner, R. H.: Am. J. Trop. Med. **9**:129, 1929.

certain pellagrins. Turner mentioned that a change in the intestinal flora might be an important factor in the etiology. The Illinois commission [11] found that the normal bacterial flora of the intestinal tract is disturbed in pellagrins, and that new forms appear, but no essential causal relationship of the organisms to the disease was demonstrated. The fungus found by Jobling and Arnold [53] to have photodynamic properties may have some significance.

The recent work of Matsumura, Kakinuma, Kawashima and others [68] in which they produced evidence that beriberi may be an infectious disease, gives further importance to the possibility that an organism is the causative factor in pellagra.

PATHOLOGIC ANATOMY

Since the etiology of pellagra has not been definitely determined, it is difficult to separate the exact pathologic changes due to pellagra. Emaciation has been reported often, but in the acute cases this is not always present. Fragility of the bones was observed by many of the early writers. Marie [69] reported fragility of the ribs in eighteen of forty-two cases. The work of Roberts [70] with use of the x-ray plate is to be commended, as he found rarefaction of the spongiosa and of the cortical layers in the bones of the hands, thus correlating the pathologic observations of the early pellagrologists. Atrophy of the muscles was found by Babès and Sion,[71] Marie [69] and Kozowsky.[72]

Integument.—The cutaneous lesions are present mainly on the surfaces exposed to sunlight, as those of the forearms, dorsa of the hands and feet and the back of the neck. Less commonly the lesions are present on the chest, face, perineum and scrotum. The symmetry of these lesions is important. Merk,[73] who described the gross changes, carefully noted the stages of erythema and of desquamation, but did not study the histology. The pathologic lesions were studied by Raymond,[74] Guard,[75] Ormsby and Singer,[76] Fiocco,[77] Kozowsky [72] and

68. Matsumura, S.; Kakinuma, G.; Kawashima, K., and others: J. A. M. A. **92**:1325, 1929.

69. Marie, A.: Lombroso's Trattato profilatico e clinico della pellagra (Turin, 1892), abridged edition, tr. from the French by Lavinder and Babcock, Columbia, S. C., 1910.

70. Roberts, S.: Pellagra, St. Louis, C. V. Mosby Company, 1914.

71. Babes, V., and Sion, V., in Nothnagel: Spezielle Pathologie und Therapie, Vienna, 1901, vol. 24, no. 2, pt. 3, p. 1.

72. Kozowsky: Arch. f. Psychiat. **49**:204, 556 and 873, 1912.

73. Merk, L.: Le manifestazioni cutanée della pellagra, Innsbruck, 1910.

74. Raymond, P.: Ann. de dermat. et syph. **10**:627, 1889.

75. Guard, F. B.: J. Exper. Med. **13**:98, 1911.

76. Ormsby, O. S., and Singer, H. D.: Report of the Pellagra Commission of the State of Illinois, Springfield, Illinois, State Printer, 1911, p. 16.

77. Fiocco: Atti d. quinto cong. pellagra ital. Bergamo, 1912, p. 305.

Babès and Sion [71] with varying results, principally because the stages of the disease are not given the importance deserved. Guard [75] correlated the changes in the skin with those normally produced by the action of sunlight, except that they are more marked. He differentiated the stages in his studies. Babès and Sion [71] found pigmentation, hyperkeratosis, hyperemia, edema and erythema, which evidently indicate stages. The pathologic changes in the skin were elucidated by MacNeal,[78] who stated that they are dependent on the stage of the disease. The early stage is represented by vascular injection and edema of the corium with a diffuse infiltration of wandering cells. Later edema and hyperplasia of the rete with regions of parakeratosis appear. Hyperkeratosis and hyperpigmentation are present as the malady advances. MacNeal [78] noted that the relationship of these processes varies according to the rapidity of the evolution and the severity of the eruption. Denton [79] summarized the cutaneous lesions as occurring in the following stages: injury, or fibrolytic stage; reaction, or dermatitis stage; repair; compensatory vascular ectasis, or erythematous stage, and the cicatricial, or atrophic, stage. This summary also makes clear the earlier reports. The fact that the various stages may be present in the same person at the same time has not been emphasized as much as it should be.

The Nervous System.—As varying degrees of pathologic changes had been found in the nervous system, MacNeal [78] believed that these lesions should be studied in relation to the stage of the disease. The pathologic reports by Babès and Sion,[71] Marie,[69] the Illinois commission,[11] Kozowsky,[72] Sandwith,[80] Sundwall,[81] Castellani and Chalmers,[82] Harris,[62] Winkelman [83] and Pentschew [84] did not adequately emphasize the stage and its relationship to the pathologic changes. Marie [69] noted atrophy of the brain in cases of long standing mental derangement. Singer and Pollock [85] did not find that the gross morbid anatomy was characteristic, although they found edema and thickening of the meninges. However, they differentiated between acute changes, consisting in chromatolysis of nerve cells, satellitosis, astrocytosis and the presence of ameboid glial cells, and chronic changes, such as fatty and fibrinoid degenerations, regressive changes of the nerve cells, chronic Nissl

78. MacNeal, W. J.: Am. J. M. Sc. **161**:469, 1921.
79. Denton, J.: Am. J. Trop. Med. **5**:173, 1925.
80. Sandwith, F. M.: J. Path. & Bact. **7**:460, 1901.
81. Sundwall, J.: Hygienic Laboratory Bulletin 106, 1917, p. 50.
82. Castellani, A., and Chalmers, A. J.: Manual of Tropical Medicine, New York, William Wood & Company, 1919.
83. Winkelman, N. W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **102**:38, 1926.
84. Pentschew, A.: Ztschr. f. d. ges. Neurol. u. Psychiat. **118**:17, 1929.
85. Singer, H. D., and Pollock, L. J.: Arch. Int. Med. **11**:565, 1913.

changes of the nerve cells, increase of glial fibers, permanent destruction of nerve fibers and a marked increase of amyloid bodies. Central neuritis was present during or soon after an acute attack. Mott [86] found degenerated fibers of nerves; these were found generally diffused and scattered throughout the white matter of the cord, but were most marked in the posterolateral and the posteriomedian columns. The cells of all of the posterior spinal ganglions showed, in varying degrees, marked chromatolysis, swelling, and disappearance of Nissl granules except at the periphery. Wherever the nervous system was examined, the cells that normally had a Nissl pattern seemed to show a change in the nature of a disappearance of the granules. Fibril changes occurred in the cells of the anterior horns and the cells of Purkinje, and also in Betz' cells, but to a less degree. Castellani and Chalmers [82] wrote that degeneration occurred in the cells of the spinal ganglions, in those of the posterior cornu and in those of Clarke's column, with Nissl bodies and fibrils disappearing in the posterior roots, in Lissauer's tract, in Burdock's column, in Goll's column and less commonly in the lateral columns. Harris,[62] who reviewed the literature extensively, stated that in the medullar and cerebral cortex areas of sclerotic changes were present, resembling those in the cord. The pyramidal cells suffered most. The changes in the spinal and sympathetic ganglions were similar to those found in the central nervous system, although usually not so marked. The early arteriosclerotic changes in the brain reported by Denton [79] were of importance, as he found them in patients under 25 years of age. He concluded that the changes in the central nervous system were not in direct proportion to the acuteness and severity of the disease, but that they were more pronounced in chronic pellagrins. His statements probably represent the truth, for many of his observations were similar to those of other pathologists, but his material was somewhat limited.

The Gastro-Intestinal Tract.—Harris [62] reported that the red and inflamed tongue often became ulcerated. Hypertrophy of the superficial lingual tissues occurred if the attacks were repeated. This hypertrophy was followed by shrinkage. Similar changes were present in the gums, lips and buccal mucous membrane. Denton [79] found that in the pharynx, tongue and esophagus the repair of the membrana propria was imperfect, and that the epithelium regenerated in a thin layer. He found that this reaction was essentially correlated with that in the skin. MacNeal emphasized that hyperemia of the intestines might alternate with a normal condition of the bowel. I later observed this change. MacNeal [78] noted that in the earlier lesions there were vascular

86. Mott, F. W.: Brit. M. J. **2**:4, 1913.

engorgement, hyperplasia of the lymphoid tissue, and edema and infiltration of the mucosa with wandering cells. In the later lesions collections of wandering cells extended from the epithelial surface into the submucosa and along the sheaths of blood vessels into the muscular coats. The inflammation of the large intestine reported by Lynch [87] was practically limited to the colon. Microscopically, the mucosa was hyperemic; the surface epithelium was degenerated and desquamated; there was an increased number of interglandular mononuclears and connective tissue cells, and there was marked degeneration of the epithelium of the glands with cystic dilation. The mouth and the neck of the gland underwent hyaline changes with occlusion by degenerated and cast-off epithelium, which he believed to be the beginning of the process leading to active inflammation of the mucosa, submucosa and other coats to thrombosis of mucosal and submucosal veins and lymphatics and to necrosis with ulceration. In chronic cases Lynch [87] found sclerosis of the small blood vessels out of proportion to the arteriosclerosis elsewhere. Denton [79] described changes such as cellular infiltrations, varying grades of acute inflammation, dilated blood vessels and changes in the sizes and shapes of surface epithelial cells, yet he did not consider them absolutely specific. Lynch [87] believed that the changes that he described in the colon were characteristic of pellagra.

Liver.—Babès and Sion,[71] de Giovanni,[88] Sundwall,[81] Crutchfield,[89] Kozowsky,[72] Denton [79] and the Illinois commission [11] reported fatty changes in the liver. A small liver was noted by Marie [69] and Harris.[62] DeGiovanni [88] stated that it remained permanently smaller after an attack. The Illinois commission [11] found inflammation of a low grade in the portal connective tissue in the interlobular septums, engorgement of the intralobular capillaries and fatty degeneration around the lobule in every case. This commission concluded that some of the specimens suggested an early stage of acute yellow atrophy or the more acute forms of alcoholic cirrhosis. Focal necrosis was noted by Denton.[79] From these reports there is indication of a toxic effect, although adequate proof of this is lacking.

Pancreas.—The reports of changes in the pancreas are few, and the changes are not characteristic. Raubitschek,[90] who carefully reviewed the literature, found no characteristic changes.

Circulatory System.—Atrophy of the heart was found by Marie [69] and by Babès and Sion.[71] Babès and Sion [71] found atheromatous

87. Lynch, K. M.: Internat. Clin. **3**:130, 1930.

88. de Giovanni: Patologia del simpatica, Milano, 1896; cited by Harris (footnote 62).

89. Crutchfield, E. D.: Arch. Dermat. & Syph. **17**:650, 1928.

90. Raubitschek, H.: Ergebn. d. allg. Path. u. path. Anat. **18**:1, 1915.

plaques in the aorta, and the Illinois commission [11] reported hyaline changes in the intima of the blood vessels. Kozowsky,[72] in his reports of arteriosclerosis, eliminated all cases in persons past middle life. When he found hyalinization and thickening of the walls of the blood vessels, changes that were especially noticeable in the walls of the smaller vessels, his careful observations could be correlated with those in old age. The work of Kozowsky [72] is important. The arteriosclerosis reported by Harris [62] might have some significance.

Respiratory System.—The pathologic changes reported, such as pneumonia, emphysema, edema, pleurisy, etc., do not appear to have any relationship to pellagra. Marie [69] noted the pulmonary tuberculosis of which frequent mention had been made by the older writers.

Lymphatic System.—Marie,[69] Tuczek,[91] Babès and Sion [71] and Sundwall [81] found atrophy of the spleen. Kozowsky [72] reported the characteristic sclerotic vessels, which were more pronounced in the spleen than in the lymph nodes. Babès and Sion [71] noted tabes mesenterica associated with pellagra, especially in children.

Genito-Urinary System.—Atrophy of the kidneys was noted by Babès and Sion [71] and Marie.[69] Kozowsky [72] related destruction of tubules and thickening of Bowman's capsule and of interstitial tissues to arteriosclerosis. No noteworthy gross or microscopic pathologic changes in the genitalia could be related to pellagra.

Endocrine Glands.—The reports of changes in the suprarenal glands, hypophysis and thyroid gland are few, and the changes could scarcely be correlated with pellagra. Susman [92] found that in the suprarenals in five cases the cortical lipoid was scant or absent, but that a secondary infection would have to be eliminated. Morse [93] reported lesions resembling chronic productive thyroiditis with the follicles undergoing a compensatory reaction. The fibrosis reported to have occurred in the thyroid gland should encourage further observations, as this gland has not been given the study by pathologists that it should have.

Eyes.—The atrophy of the choroidal pigment found by Rampoldi [94] and the early cataracts reported by Welton [95] might be related to the early changes in senility. Elimination of older patients would have added value to the observations of Welton.[95]

---

91. Tuczek: A Dictionary of Psychological Medicine, Philadelphia, D. H. Tuke, 1892; cited by Marie (footnote 69).

92. Susman, W.: Tr. Roy. Soc. Trop. Med. & Hyg. **24**:23, 1930.

93. Morse, P. F.: J. Lab. & Clin. Med. **1**:217, 1916.

94. Rampoldi, R.: Ann. di ottal. e clin. ocul. **14**:99, 1885.

95. Welton, C. B.: J. A. M. A. **53**:1636, 1909.

SUMMARY

A scrutiny of the literature published during the last thirty years reveals the prominence of the theory that a deficient diet is the cause of pellagra (which is an outgrowth of the conception that the disease comes from the consumption of spoiled maize). The theory that pellagra is caused by a deficient diet has advanced recently to the theory that a deficiency of "vitamin G" is the cause. At present evidence accumulates that vitamin G is composed of two factors. Whether iron or cane sugar is related to the etiology is a question for the future. The significance of photodynamic action in pellagra still has its place, because of the seasonal variations noted in lesions of the experimental animals suffering from lack of the antipellagric vitamin; also because a fungus having fluorescent properties has been isolated from the stools of pellagrins.

Pathologic changes similar to those in senility are found in pellagrins. Erythema, edema, hyperkeratosis and hyperpigmentation of the skin, degenerative changes in the nervous system, and hyperemia, inflammation and repair of the upper gastro-intestinal tract and of the colon are the most important lesions in pellagra.

Since the exact etiologic factor in pellagra is still questioned, difficulties arise in the interpretation of the pathologic changes due to the disease.

THE ETIOLOGY OF CANCER

I. TISSUE TRANSPLANTATION AND PARASITISM *

H. E. EGGERS, M.D.
OMAHA

In the following general and more or less critical review of the subject of the etiology of cancer, no claim is made for completeness. Even if it were desirable to include references to all that has been contributed to the subject, the publications are too numerous and too scattered to permit the hope of such an absolutely complete survey. I hope, however, and trust, that few articles of major importance have been omitted here.

Owing to the unavoidable length of a summary even as condensed as this, it has become necessary to divide it into four parts. The first deals with the results of tissue transplantation in their relations to cancer, and with the problem of parasitism in cancer. The second will deal with the general subject of irritation and cancer. A third will take up various miscellaneous features of the etiology of cancer—its relations to neurotrophic changes, to nuclear aberration and to heredity, and the factors of general or local character affecting the growth and survival of already initiated tumors. The fourth and final part will deal with metabolism in its relations to the etiology of cancer.

It is not proposed to discuss here, except incidentally to facts as revealed by experimental study, the more or less classic histogenic theories of the origin of cancer; while they represent brilliant feats of deductive reasoning, they are seldom of such character as to admit of experimental control, and the expanding knowledge of the phenomena of cancer development derived largely from experimental study bids fair to supplant them entirely.

TISSUE TRANSPLANTATION

Heterologous Transfer of Tumor Tissue.—To the extent that the earlier stages of the etiologic study of cancer were a matter of experimental investigation, as aside from speculations based largely on morphologic observation, this work was devoted almost wholly to the search for a possible parasitic cause. Even the earlier experiments on the

* Submitted for publication, April 16, 1931.
* From the University of Nebraska College of Medicine.

transferability of cancer had in the background the idea of causation by transfer of a parasite rather than of the cancer itself. As early as 1840 Langenbeck reported that 60 days after the injection of human cancerous fluid into a dog the animal was found to have tumor nodules in the lung. In 1843 Klencke averred that he had confirmed Langenbeck's work, without, however, publishing any details. Somewhat similar results were reported by Lebert in 1851, but in 1867 he and Wyss were unsuccessful in transferring human tumors to rabbits or to guinea-pigs; in the same article they described the appearance of local tumors in the neck of a dog after the injection there of fluid from an ulcerated human esophageal cancer—lesions which, from their description, were almost certainly of acute inflammatory character. In that same year Goujon alleged that he had succeeded in transferring a human testicular cancer to the white rat, in which the observed tumors had no relation to the site of inoculation, and to the guinea-pig. In 1874 Quinquand found in a fowl into which he had injected a small quantity of a cancer of human liver multiple pulmonary masses, which he regarded as cancerous. Thiroloix in 1892 observed palpable masses in the abdomen of a rabbit which had been inoculated intraperitoneally with mammary cancer from a white mouse; Francotte and de Rechter the same year believed that they had produced cancer in mice by the repeated injection of human cancerous material, and in the following year Mayet observed in a rat the appearance of multiple visceral cancers a year after the injection of fluid from glycerinated and macerated human cancers into a number of those animals. He later (1901 and 1905) added to this similar reports of additional results, with alleged success in 5 of 52 attempts, and 4 apparent successes with human material introduced into dogs. In 1894 Boinet reported that he had been successful twice in transferring human cancer to the rat, and once to the rabbit, in a total of 60 attempts. Jürgen in 1896 observed generalized melanomatosis transmissible by homotransplantation in rabbits inoculated with human melanotic tumor, and in 1898 Queyrat found that pigmented intraperitoneal masses were formed in macacus monkeys after similar inoculation. Dagonet in 1903 believed that he had successfully inoculated an epithelioma of the human penis into the peritoneal cavity of the white rat, in which, 17 months later, he found disseminated intra-abdominal lesions, which he stated to be of the same type as the injected tumor. Together with Mauclaire he reported in 1904 the successful inoculation of human rectal adenocarcinoma into the rat, with the development of tumors, though of dissimilar type, in that animal which were transplantable for another generation.

Of these earlier alleged successful transfers of cancer from one species to another, none can be regarded as of undoubted authenticity. Some of the observed lesions were possibly cancerous, but, as was

believed by Virchow of Langenbeck's tumor, of spontaneous rather than induced origin. And some with even greater certainty were masses of inflammatory character, as may occasionally be inferred from the descriptions given by the experimenters themselves.

In 1905 there appeared a report by Lewin of what is by far the best authenticated and most carefully considered of the various attempts at heterotransfer of a tumor. Two months after the introduction into the peritoneal cavity of a dog of material from an unusually malignant human ovarian cancer the animal showed local masses of a morphologic type that was not definitely neoplastic, but that differed from the usual granulomas in that the tissue was inoculable into other dogs. Lewin was unable to classify the lesion exactly, but in 1907 he reported the development of inoculable granulation tissue about a bit of human cervical cancer embedded in the white rat.

Of more recent work, there is the report by Robertson, in 1909, of the appearance of malignant tumors of miscellaneous types in 7 of 30 mice fed with exudative material from human cancer. In 1911 Gargano reported a number of cases of alleged production of tumor in mice after inoculation with human material, the lesions, according to him, being originally carcinomatous, later changing to sarcoma—the inference being that they were granulomas. Strauch in 1913 believed he was successful in transferring a very malignant mouse carcinoma to 50 per cent of his rabbits, as well as in the transfer of a human cancer to a macacus monkey. But a comprehensive repetition of his work by Apolant and Bierbaum led them to the conclusion that the lesions observed by Strauch were inflammatory. In 1923 Nather reported the transfer of cancer from mouse to rabbit by the repeated injection of emulsions of tumors, and Heidenhain in 1928 stated that in mice into which autolysates of the malignant tumors of man, cattle and mice had been injected there was an unduly high frequency of tumor development. The tumors were of diverse character; as a rule there was no relationship of the site of the tumor to that of the injection, and the rate of incidence was not sufficiently high to exclude the possibility of spontaneous occurrence.

Actually there is a much greater volume of evidence to show that heterotransfer of tumors does not take place, except in certain circumstances that infringe rather slightly on the bounds of tissue specificity. Among those reporting unsuccessful attempts at the transfer of human cancer to various laboratory animals are Fischer in 1882, Senn and Senger in 1888, Klebs in 1890, Morau in 1891, Fischel, Duplay and Cazin in 1892, Kopfstein in 1893, Curtis in 1899, McConnell in 1908, Vidal in 1909, Bartkiewicz and Leotta in 1910, Williams in 1911, Fischel in 1922, Kurtzahn in 1926 and Tinozzi in 1929. The foregoing list of investigators who reported failures in the attempted heterotransplantation of

tumors is by no means exhaustive—numerous earlier efforts, not cited here, are given in reviews by Pilliet and by Vischer. Suffice it to say that the altogether predominant evidence favors the view that the successful transfer of malignant tumors, with certain special exceptions, may be accomplished only in circumstances that permit the continued survival and growth of the tumor cell itself, and of course in these circumstances the transfer of the tumor to a new host is actually that, and not the transfer of a causative agent of possibly parasitic character. Careful scrutiny of the cases recorded in the foregoing paragraphs which do not accord with this view will show, practically without exception, that the allegedly positive results cannot be accepted without some skepticism. In many the tumor-like lesions were almost certainly of inflammatory character; in others the growths would appear to have been spontaneous tumors, and the very plenitude of positive results reported by some experimenters are so unlike the results of most other investigators as to make them of doubtful authenticity.

Among the special exceptions mentioned must be included a group of dog tumors that show certain peculiarities in the way of transmissibility. There have been reported a considerable number of transferable tumors of this animal, including some of the earliest successful efforts at transplantation of tumors. Smith and Washbourn reported in 1897 the occurrence in dogs of tumors, apparently round cell sarcomas, which were readily propagated by implantation and which were transmissible by coitus. In 1902 White, with another instance of this type, reported that the tumors were lymphosarcomas, and again observed transmission by coitus. In 1904 Sticker described a similar tumor with a high degree of transmissibility by inoculation, and in 1906 Beebe and Ewing reported on another that combined the features of high inoculability and venereal transmission. In that year Sticker found that his tumor was inoculable into foxes, although not into a miscellaneous series of utterly unrelated animals. That this was not a matter of cellular transfer was suggested by Wade in 1907, who regarded the disease as a general infection with localized lesions of sarcomatous type; while the virus was almost certainly intracellular, it was not filtrable, a finding that was confirmed by Beebe and Ewing. In 1909 Sticker observed in the breast of a bitch that had been inoculated with one of these sarcomas (in this case of spindle cell type) the development of a slowly growing, but metastasizing, adenocarcinoma; and in 1912 von Dungern was able to show definitely that in the transfer of these sarcomas to foxes cellular implantation was not concerned, by the failure of the induced fox tumors to produce dog antibodies after injection into other foxes—unlike the result that followed the injection of the original dog tumor tissue.

Homologous Transfer of Tumor Tissue.—Although, in general, experimental heterotransplantation has yielded negative results, the situation is of course quite different as concerns the transfer of cancer within the host species. Even before the successful artificial transplantation of tumors, clinical observation had shown that such transplantation took place within the individual, even if the phenomena of metastasis were not sufficient to establish this. Natural transplantation by implantation was reported by Virchow in 1863, Kaufmann in 1879, Nicasse in 1883, Kraske and Lucke in 1884, Geissler in 1893, Claude and Pilliet in 1895, Cabot in 1901 and Wilmanns in 1904, and by intentional surgical procedures by Hahn in 1888 and Cornil in 1892. From these, it could be surmised logically that transfer from one animal to another sufficiently akin should be possible, and although the limited number of attempts at this with human beings—such attempts were reported by Senn in 1888 and by Kurtzahn in 1926, each using himself as the subject—were unsuccessful, as early as 1876 Novinsky reported the transfer of a cancer of the nose from one dog to another, with doubtful success in the second generation. A similar attempt reported by Mathis in 1885 was not unquestionably successful, but in 1889 both Wehr and Hanau were able to secure successful transfers; the one with a squamous cell carcinoma of the dog, the other with a similar tumor of the rat, which he transplanted for 3 generations. In the next year von Eiselberg reported the transfer of a fibrosarcoma from one rat to another, and in 1891 Morau announced the transplantation of a mouse carcinoma, which by 1893 had apparently attained 17 generations. Firket in 1892 succeeded with a rat sarcoma, and Velich in 1898 with a similar tumor, in this case for 9 generations. Although these earlier successes did not attract widespread attention, the almost simultaneous work of Loeb, with a sarcoma of the thyroid gland of the white rat, which he was able to propagate indefinitely, and of Jensen, with a carcinomatous tumor of the mouse, which by 1903 he had transplanted for 19 generations, established beyond question the feasibility of homotransplantation of malignant tumors. But from the point of view of the etiology of tumors, neither these nor the numerous subsequent successful transfers have been of direct value, since the phenomena of transplantation are essentially those of artificial metastasis of already established tumors. The extent to which they have been of value in this connection has been to supply a constant and accessible source of tumor material and to afford some knowledge of the conditions permitting tumor growth.

Strictly speaking, tumor transplantation is not entirely limited to animals of the same species. Occasional cases are not subject to this restriction; von Dungern and Coca found a sarcoma of the hare which could be transplanted into rabbits without loss of its original hare

specificity, and Fujinami and Hatano, a fowl sarcoma that would survive in the duck. Flexner reported the temporary growth of human teratomatous tissue inoculated into monkeys, and Putnoky very recently published an account of the transfer of the Ehrlich mouse carcinoma to the rat, with successful retransfer back to mice. The successful growth of tumor tissue in foreign embryos, as reported by Murphy, Kiyono, Kawakami and Sueyasu, Stevenson, Bullock and Gheorgiu, does not materially affect the problem, as Murphy showed that such growth ceased in the later periods of embryonic life, when tissue specificity appeared. This same lack of specificity evidently applies to the heterologous implantation of tumors into brain tissue, as reported by Murphy, and into the eyeball, as observed by Hegner. Instances of heterologous transfer after modification of the new host, as reported by Roskin for fowl sarcoma introduced into the mouse after reticulo-endothelial blockage, are presumably to be interpreted, too, as instances of survival of the introduced tumor cells and their descendants. Other, less definite means of modification of the new host have been attempted, in some cases with alleged success. Funk in 1915 was able to implant an inoculable chondroma of the mouse into the rat after feeding the latter with the tumor tissue prior to inoculation, and Nagayo and Wago in 1921 also reported the successful heterotransplantation, though to a limited degree, of tumor after preliminary preparation of the new host. Keysser in 1920 reported the appearance of a sarcoma in 1 of a number of mice inoculated with the emulsion of a rapidly growing human testicular tumor, after they had been given injections of autolysates of human sarcomas. And Flaszen and Wachtel reported that human cancer could be successfully implanted in white mice after preliminary alkalinization; but in this case the photomicrographs are particularly unconvincing.

Transplantation of Embryonic Tissues.—It may be well to include at this point the attempts at causation of tumors by the introduction of embryonic tissues, with their obvious relationship to Cohnheim's hypothesis. Such implantation has been attempted by numerous observers, Leopold in 1881, Lwoff in 1883, Zahn in 1884, Fere in 1897, Traina in 1902, Fraenkel in 1903, Wilms in 1904, Petrow, Rössle and von Hippel in 1906, Bogoljuboff in 1908, Askanazy in 1907 and 1909, Neuhäuser in 1909 and 1911, Shattock, Seligmann and Dudgeon in 1909-1910, von Tiesenhausen in 1909, Freund and also Rous in 1911, Gargano in 1914, Ikematsu in 1923, Wereschinski in 1924, Skubisrewski in 1925 and 1928, Ide and Loewenthal in 1928. The result of all of these attempts, with occasional exceptions, was the occurrence of growth for a limited period, followed either by persistence without progression or by eventual regression. Among the exceptions are those of: Petrow, who found that the growths obtained in his

animals showed some slight inoculability into other animals of the same species; Askanazy, who observed the development in an experimental teratoid of a sarcoma inoculable for 1 generation only, and according to his later report, of teratoids which could themselves be transplanted for 2 or 3 generations; Neuhäuser, who after the introduction of fetal adrenal tissue into the kidney of an adult rabbit, observed the appearance of a tumor first regarded as a hypernephroma, later as adenomatous; von Tiesenhausen and Skubisrewski, who both saw in some of their teratoids sarcoma-like inclusions, which were, however, without other evidence of progressive growth; and Wereschinsky, who believed that he had obtained a spindle cell sarcoma in 1 case after the implantation of fetal guinea-pig tissue in the adult animal. Belogolowy, too, observed what he regarded as a sarcomatous growth of implanted amphibian embryonic tissue, a belief in which he was supported by Roux. But repetition of his experiments by Piette, Anders, Bierich and Teutschlaender led all these to the conclusion that to the extent that proliferation occurred it was of granulomatous character. The mass of evidence would seem to be sufficient to establish the fact that embryonic displacement alone is not sufficient to cause malignant growth, at least with displacement such as may be achieved experimentally. The exceptional instances in which cancer has followed such experimental displacement have thrown no light on the problem of the causation of the tumor, except in some more complicated experiments, to be discussed later, in which to the displacement was added accessory action of one sort or another.

Transplantation of Adult Tissues.—Similarly, a number of experiments involving the transplantation of adult tissues have been undertaken in an effort to check Ribbert's hypothesis of postembryonic displacement as a factor in the genesis of tumor. Even before the publication of Ribbert's hypothesis, Schweninger in 1886 had reported that transplanted epithelium showed only restricted growth; Levin in 1901 likewise observed some growth of transplanted skin, but not of other tissues; Marnoch in that year, Nichols in 1904-1905 and von Lamezan in 1913 observed at most only limited proliferation after transplantation. Only one or two reported experiments stand in contradiction to these findings. Lack in 1900 inserted ovarian scrapings of rabbits into their peritoneal cavities, and in 1 of 2 surviving animals found a generalized implantation of epithelium throughout the peritoneum, with growths in the liver, pleura and lungs. And in 1912 Stieve found that in 1 of 46 guinea-pigs in which the effort was made to transplant the granulation tissue produced in response to the injection of kieselguhr, the tissue on implantation was capable of sustained, independent and infiltrative growth.

PARASITISM

Protozoa.—While the earlier attempts at the transfer of cancer were based on the idea of demonstrating its parasitic cause, numerous more direct efforts at the discovery of a conjectured parasite were being made. Among these were the numerous attempts to discover protozoan organisms, and the earlier literature on the etiology of cancer abounds with the claims of those who believed that such parasites had been discovered. For the most part, these claims were based wholly on morphologic observation. Unusual inclusion bodies in cancer had been observed by Müller as far back as 1839, by Virchow and by Bruch in 1847, and by Hannover in 1852. Virchow, at least, interpreted these as degenerative inclusions, and it was not until 1888 that Pfeiffer announced the belief that among them were forms that could be regarded as protozoa. In the following year, Darier, Albarran, Malassez and Thoma reported what they regarded as sporozoa or coccidia in human cancers, with as a rule a claim for their etiologic relationship to the disease. Darier based his claim largely on a study of Paget's disease of the breast, and in 1890 Hutchinson and Wickham believed that they were able to substantiate his claim. In this year other reports of supposed protozoa in cancer were made by Hache, van Heukelom, Sibley, Sjöbring, Vincent and again by Pfeiffer. Not all of these reports were accompanied by claims of causal association—this is true throughout this entire field of cancer research—but enough positive statements were made to excite a considerable volume of adverse comment. Cazin, Eberth, Klebs and Schütz were among the first to deny the parasitic nature of these inclusions, and Borrel particularly insisted on their degenerative character. Possibly as a result of these views two reports of protozoon-like inclusions made in 1891 by Bowlby and Stroebe were reserved in their interpretations, as was the case with a similar report made by Kürsteiner in 1893, and their identification of the bodies as parasites was at most only tentative. In the meantime there was an increasing mass of adverse opinion, Fabre-Domergue, Boeck, Firket, Kanthack, Piffard, Pilliet, Ribbert and Steinhaus all taking this view. Since Delepine had suggested that the mildly stimulant action of many protozoa would well serve to explain the phenomena of cancer, Shattock and Ballance attempted cultivation experiments with cancerous tissues, along with the inoculation of cancers into lower animals, as well as of coccidia into rabbits, with entirely negative results. During 1892 the discussion continued unabated, Boyce, Cazin, Fabre-Domergue, Flexner, Karg, Müller, Kosinsky, Raum and Virchow maintaining that the disputed inclusions had their origin in phagocytic or degenerative processes, and Delepine becoming more doubtful of their micro-organic character. Steinhaus, on the other hand, was disposed to admit the parasitic nature

of some of the inclusions, and Foa, Malassez, Metchnikoff, Soudakewitch, Sawtschenko, Ruffer, Walker and Podwyssozki either reported the identification of protozoan parasites or claimed this character for organisms previously described. During the next few years the negative view was argued by Boyce and Giles, Cazin and Duplay, Claessen, Gibbes, Hebb, Ohlmacher, Pianese, Petersen, Rosenthal, Snow, Fabre-Domergue, Cornil, Steinhaus, Ribbert, Adler and Borrel, and with certain reservations by Power, Massari and Ferroni. But at the same time renewed claims were being made by Burchardt, Cattle, Clarke, Galloway, Korotneff, Pawlowsky, Ruffer, Ssudakewitsch, Foa, Kahane, Kurloff, Nepveu, Steven and Brown, Vedeler, Smith, Power, Busquet, Bosc, Gaylord, Eisen, Sjöbring, Schüller, Jürgen, von Leyden, Jaboulay and Feinberg. With almost all of these alleged parasites, the only evidence of their existence was morphologic observation, and occasionally this was the subject of severe criticism—the organisms of Schüller, for instance, being identified, perhaps with justice, as particles of cork. However, Gaylord believed that the inoculation of his protozoa into guinea-pigs was occasionally followed by the appearance of cancerous lesions, and Sjöbring that similar results followed when his parasites were injected into white mice. Even later claims have been made for protozoan parasitism in cancer by Saul, Robertson, Jaboulay, Awerinzew, Elmassian, Clarke, Koch and, as lately as 1928, by Krug. Later exponents of the adverse view have been Nösske, Honda, Calkins, Borrel, Apolant and Embden, Klimenko, Bastian, Rosenberger, Unna, Greenough, Farmer, Moore and Walker, Blum, Grünbaum and Podwyssozkoi, who in 1909 acknowledged the degenerative character of organisms previously regarded by him as parasites, Brown, McDonagh, Lewis, McConnell and Lang, Deetjen and Machiarulo. While there was no particular harmony in the ideas of just what the inclusions might be, all the aforementioned investigators were insistent that the identification of the inclusion bodies as parasites was incorrect or at least conjectural, and their etiologic significance even more so.

More carefully considered than the attempts at identification of a parasite almost solely on morphologic grounds was the work of O. Schmidt, who in 1903 reported the observation in cancer of a sporulating parasite, with ciliated and ameboid stages, which in his experience could be grown on strains of *Mucor racemosus*. In later publications he announced that inoculation of infected colonies of *Mucor* into laboratory animals was followed by the appearance of malignant tumors—sarcomas in rats and mice and 1 carcinoma in the mouse. In addition, he found that injection of killed cultures had an immunizing effect against implanted sarcomas in rats. But Schuberg in 1906 disagreed as to the protozoan character of the alleged organism; Baisch in 1908

observed only one tumor in 70 survivors of 100 mice inoculated with Schmidt's organism, and quite failed to get any evidence of immunizing action; von Wasilewski and Wülker in 1912 could not find the protozoon in strains of *Mucor* obtained from Schmidt, nor in attempts of their own at its isolation; nor could they produce tumors in animals by experiments reproducing those of Schmidt as closely as possible. On the other hand, Rehorn in 1928 cultivated strains of *Mucor* from 2 human tumors, and in 70 rats inoculated with these observed the appearance of 2 cases of tumor.

Alleged successful causation of malignant tumors in animals by the injection of supposedly protozoan organisms was also reported by Walker in 1911, and by Flynn in 1922. The former worked with an organism obtained from the earthworm, which he regarded as the intermediate host; his photomicrographs show what appear to be granulomas. As to Flynn's work, the report is not sufficiently detailed to permit discussion. Von Calcar reported in some detail experiments to prove the protozoan origin of cancer: observations of proliferative growth in the livers of dogs, occurring only about such individual trematodes as harbor protozoa *(Metorrchis truncatus)*; lessened incidence of tar cancer in mice shielded from possible protozoan infection; induction of cancers in achylic dogs by allegedly infected material, etc.

The discovery that myxameboid organisms, parasitic in plants, were capable of exciting these to redundant tumor-like growth, served as a stimulus to the identification of similar organisms as the cause of tumors in animals. The protozon reported by Pfeiffer in 1890 was regarded by him as analogous in appearance to *Synchytrium taraxaci*, and that of von Leyden was compared with *Plasmodiophora brassicae*; Robertson and Wade and Fink in 1905 fancied a like similarity between apparent observed organisms and that parasite, as had Gaylord in 1902. Behla, who adduced the parasitic nature of cancer on indirect grounds, was inclined to implicate organisms of this type. However, although Podwyssotzkoi in 1900 got what he regarded as giant cell sarcomas in rabbits and guinea-pigs after their inoculation with infected leaves, in 1905 Löwenthal reported negative results from inoculation experiments, and Saul in 1906 obtained at most epithelial and connective tissue hyperplasia suggestive of chronic inflammation. Von Prowazek, after close study, was unable to find any similarity between the suspected cancer inclusions and *P. brassicae*.

The assertion made by Adamkiewicz in 1893 to the effect that the cancer cell itself is a foreign protozoan parasite, which was persistently advocated by him as lately as 1911, evoked some of the harshest criticism (Kinscherf and Bartsch, Paltauf and von Hansemann) that appears in the annals of cancer investigation. A similar view was advanced by Butlin, but received no wider acceptance. The idea that the

cancer cell itself is a parasite, not necessarily of foreign origin, has been advanced with some frequency—by Bard, Debove, Raymond, Tyzzer, Walsh and Critzmann, the latter with the additional conception that its origin results from the inclusion of one impregnated ovum within another. The conception of the cancer cell as a parasite, without any suggestion of how that parasitism is achieved, simply serves as a brief and more or less accurate characterization of its behavior, and is of course without bearing on etiology.

This statement does not apply to the theory of Kelling, who regarded cancer as the result of implantation in the organism of foreign embryonic cells, the character of which he believed he could identify by suitable immune reactions; he reported the experimental induction of tumors, though in a small proportion of his animals, by methods which he regarded as confirming his views. In an analysis of Kelling's work by von Dungern, it was pointed out that the immune reactions which he reported were much more plausibly explained as being of nonspecific character. Lubarsch, although he could not accept Kelling's theory, believed that in some of the latter's experiments there was evidence of transfer of tumor-inducing substances, but Ribbert refused completely to credit Kelling's reports of apparently successful induction of tumors.

Bacteria and Other Vegetable Parasites.—Almost coincident with the reported occurrence of protozoan parasites in cancerous growths were a number of efforts to implicate various bacteria as their cause. In 1887 Scheurlen described the isolation from 10 cases of human cancer of a spore-forming bacillus with which he believed he could cause carcinoma of the breast in dogs. Rappin, in the same year, similarly obtained a diplococcus, which in 1 instance caused what he described as a cancer of the breast in an inoculated rabbit; Schill reported the occurrence in both carcinomas and sarcomas of bipunctate organisms, for which, however, he made no etiologic claim. Meanwhile, Ballance and Shattock, although professed believers in the infectious origin of tumors, reported that in the great majority of cancers studied by them the bacteriologic observations were consistently negative. In 1888 Freire believed that he could confirm Scheurlen's findings, and even claimed to have anticipated them; Francke, too, agreed with Scheurlen as to the presence of the bacillus in human tumors. But Senger, Pfeiffer and Baumgarten, all working with this bacillus, were unanimous in regarding it as a mere contaminant, basing their conclusions on its cultural characteristics, on the fact that it appeared not infrequently as a contaminating organism on their culture mediums, and on its only occasional presence in tumors which were almost without exception ulcerated. Makara reported that systemic bacteriologic study of cancers only rarely showed the presence of bacteria, even as

accidental invaders, while Bernabei, who recovered from tumors organisms similar to those described by Schill, found that their only effect on inoculation was the production of a rapidly fatal marasmus and cachexia. Rosenthal, and a little later Sanquirico, after studies of Scheurlen's bacillus, denied that it could have any causal relationship. That organisms could easily occur in cancer without having necessarily any bearing on its cause was indicated by Gley and Charrin, who found a rabbit tumor heavily infected with *Staphylococcus aureus.* Nevertheless, in 1890 Koubassoff found in human cancers a short bacillus which on inoculation into laboratory animals caused disseminated nodular growths. A second report was made in 1891 by Ballance and Shattock of negative bacteriologic observations in cancer, and since that time the claims for a discovery of a specific bacterial agent have been relatively infrequent. But a number of statements have been made asserting that the cause of cancer was to be sought in higher vegetable organisms. Von Niessen in 1894, Braithwaite in 1895, Bra in 1898 and Chevalier in 1899 found mycotic organisms in cancer which were in part claimed to have a causal relationship, Chevalier believing that this view was supported by inoculation experiments, which Fabre-Domergue and Richardson were unable to confirm.

The attempted identification of saccharomycetes or blastomycetes as the cause of cancer has given rise to such an extensive literature as to necessitate its discussion as a separate item. To continue the more general aspect of the subject, a report by Monsarrat to the effect that he had recovered from cancers a pleomorphic organism that would produce in guinea-pigs tumor-like growths, on being reviewed by a committee of the Pathological Society of London, brought the verdict that these were of granulomatous character. In 1905 Hoffmann announced the finding in ulcerated cancers of spirochetes, but although this was confirmed by Loewenthal in 1906, Beebe and Gaylord in 1907, and by Deetjen and Simmonds in 1908, the significance of their relationship to the ulceration was quite generally recognized, only Gaylord suspecting that they might play a causative rôle. Deetjen, indeed, had found them present in nonulcerated tumors in the mouse, but he was able to find a strain of the same tumor in which they were absent, and to show that in some unsuccessful attempts at transfer of tumors, the spirochetes might be inoculated independently; in 1919 Mueller showed that it was possible to destroy the spirochetes in these tumors without affecting the latter.

The *Micrococcus neoformans* of Doyen—as its name indicates, a coccoid organism in which he believed that he had found the cause of the disease, with apparent production of cancers in rats following its inoculation—met with the same fate as previously announced bacterial causes. Karwacki regarded it as a secondary invader, and Letulle

reported that the alleged tumors caused by it were in reality of inflammatory character. In 1914 Minett identified this organism with *Staphylococcus albus,* which is frequently found in the deeper tissues. Much of the interest in *M. neoformans* lies in the explanation by Doyen of the mechanism of its production of tumors—the provocation by it of excessive and abnormal phagocytic activity, which, leading to intranuclear parasitism, gave the cells the property of unrestricted growth.

In 1908 Robertson reported that he found in cancer rod-shaped organisms, which, however, he regarded as a stage in the life cycle of a conjectured protozoon of alleged causal relationship. In 1921 the same writer implicated large diphtheroid organisms as the probable cause. Leyton in 1916 claimed to have isolated from rat tumor a filtrable streptothrix which on repeated injection into rats caused in them inoculable sarcomas, and in 1920 King found in cancer a fungoid organism with a complex life cycle, for which he also claimed positive results on inoculation. In 1921 Nuzum reported that in cultures from transferable mouse cancers he obtained a coccoid organism that produced tumors in inoculated mice; in 1925, with a streptococcus isolated from malignant tumors, he observed that following its inoculation into the breast duct of dogs, there was a development of cancer there in 2 of 5 attempts, 1 of the dogs dying with generalized cancerous metastases. Also in an elderly man, who had previously had an epithelioma, a similar tumor appeared after repeated inoculation with this organism. Nuzum's earlier work was checked, unsuccessfully, by Kross.

The etiologic relationship of pleomorphic bacteria to malignant tumors has been the subject of a number of claims. Young in 1921 stated that tumor developed in 10 per cent of mice inoculated with such an organism. Glover, and Loudon in confirmation of him, made similar claims, which Kolmer, Harkins and Saleeby, after repeating Glover's experiments with scrupulous observance of his technic, were unable to verify. Purpura isolated from cancer a pleomorphic organism for which, however, no etiologic relationship was suggested, and Lieske as recently as 1928 implicated such an organism as the cause. Nissle in 1927 stated that in mice he had caused cancer by the injection of molds or their filtered broth cultures; Lumiere and Montoloy, also in that year, failed in 45 tumors to find any organisms that were not almost certainly secondary invaders.

Reference has been made to the suggested identification of blastomycetes as the cause of cancer. The stimulus to this was furnished by a report by Busse to the effect that these organisms had been found to cause a human lesion which he first believed to be a giant cell sarcoma of bone, although in a later report he recognized it as a chronic inflammatory lesion. Even before this, Russell had described his "fuchsin bodies," which he had interpreted as yeasts, though Klien, Dean and

Councilman had announced that in their opinions they were, like the alleged protozoa, simply the products of degenerative changes; Bergonzini, while disposed to concede their living character, questioned any possible relationship to the cause of cancer. In 1895, the year of Busse's amended report, Sanfelice, Aievoli, Rossi-Doria, Kahane, D'Anna, Curtis, Roncali and Corselli reported the isolation of organisms of this type from tumors, and in some cases the causation by these of tumors in laboratory animals. Similar claims were subsequently made by Binaghi, Mirto, Hare and Harris, Plimmer, Leopold, Wlaeff and Fabry and Trautmann. Sanfelice believed that Russell's bodies could be identified as of the type of the saccharomycetes which he regarded as the cause of tumors, and Roncali announced a similar identification of Korotneff's parasites. But Mafucci and Sirleo, although believing many malignant tumors to be of infectious origin, recognized the inflammatory character of blastomycotic lesions, as did Frothingham, Bonome, Galeotti, Henke, Nichols and Petersen and Exner; Lubarsch and Franchetti reported negative results following the inoculation of animals; Sternberg regarded the alleged blastomycetes in tumors as degenerative products; Lecount and Greenough made the same identification of the alleged organisms described by Plimmer, and Spirlas found that Plimmer's bodies could be produced by the injection of a considerable number of diverse agents. Alessandri in 1903 reported that cultivation attempts with 33 tumors yielded consistently negative results as regards blastomycetes, although that they were occasionally present was shown by McCampbell, who recovered them from a spontaneous tumor of a wild mouse, but was unable to find any tumor-producing action by these in experimental transfer. Loeb, Moore and Fleisher only exceptionally found yeasts in malignant human tumors, and their attempts at the causation of tumors by yeasts yielded only inflammatory lesions. Of late years the attempted identification of the blastomycetes as the cause of malignant growths has been almost completely abandoned, although Pentimalli in 1912, having observed local hyperplasia of tissue in fowls into which endotoxins of these yeasts had been injected, expressed the belief that infection with them may be a contributing factor; Roncali in 1914 continued to regard them as one of a number of parasites acting as causes, and as lately as 1929 Sanfelice believed that he was able to observe amelioration of human cancers treated with preparations of blastomycetes. A reexamination of Russell's fuchsin bodies by McConnell and Lang in 1921 led them to conclude that these bodies were the result of cytoplasmic degenerative changes. There are few organisms that are more definitely associated with hyperplasia of tissue than are the pathogenic yeasts, and it was undoubtedly the irregular and excessive epithelial overgrowth which these infections occasion that led to the conclusion

that they were the cause of cancer, despite the absence of independent and unrestricted, and of remote, growth.

Bacillus Tumefaciens: A discovery of association between certain bacterial infections and the inception of tumors, in which the exact relations are still far from clear, resulted more or less directly from a study of crown galls in plants. A similarity between plant galls and animal tumors was noted even in 1887 by Paget, and Packard again called attention to it in 1911. In an article published in 1912, E. Smith emphasized the resemblance of crown galls to malignant tumors, especially as regards the features of invasive and secondary growth, and a few years later he was able to show that these lesions of plants were caused by infection with a bacterium which he termed *Bacillus tumefaciens*. The close analogy of the plant and animal tumors is still disputed. Levin, who in 1920 accepted the analogy, in 1922 pointed out that unlike the neoplasms of animals, those of plants possessed tissues capable of complete differentiation. Blumenthal, along with Hirschfeld, also expressed doubt of the similarity, and Stapp in 1927 expressed the belief that the crown galls were more properly to be regarded as granulomas, while Vasiliu regarded them as more analogous to benign tumors of animals. Nemec in 1928 also viewed them as granulomas, as did Scaglione and Hamdi in 1929. Agreeing with Smith in regarding them as plant cancers was Magrow in 1924, Auler in 1927 and, as will appear shortly, Blumenthal in a number of his later reports. In 1920 Smith was able to secure tumor-like hyperplasia in plants by the application of dilute alkalies and acids, by the introduction of foreign organisms and by partial asphyxiation—results which would appear to suggest a granulomatous reaction—a view further sustained by the causation in plants of lesions similar to the bacterial crown galls by the injection of 1 per cent lactic acid solution, as reported by Blumenthal and Meyer in 1923.

A year later Blumenthal, Auler and Meyer reported that from 12 of 30 human tumors they had succeeded in isolating organisms which on inoculation into animals induced malignant tumors; the latter finding was confirmed by Pickhan. Especially significant appeared to be the fact that the organisms could not be recovered from the induced tumors. In spite of certain cultural differences, it was at first believed that the several bacterial strains isolated were closely related to each other and to Smith's *B. tumefaciens*, a belief which was not substantiated by later work, especially by that of Reichert, who demonstrated decided differences in the several organisms, not only in cultural characters, but also in regard to toxicity and immunity reactions, this last fact being later confirmed by Becker. It was the usual experience that *B. tumefaciens* of plant origin was without effect on animals—among the published experiments to this effect being those of Teutschlaender and of Borghi

and Luzzatto. Those of animal origin, on the other hand, were frequently active in plants, as shown by Kauffmann with a bacillus isolated from mouse cancer, by Fejgin, Epstein and Funk, and by Bechhold and Smith—at times even when their action on animals was no longer manifest.

That the tumefacient action of these bacteria is apparently not dependent on the organism itself is indicated by a number of observations. So in 1919 Blumenthal observed that accidental contaminants at times became possessed of this effect, a result which he was not able to achieve intentionally. The outstanding differences between various organisms of the tumefacient group led Reichert to seek a common property in a filtrable virus which he believed they harbored, a view which was later shared by Blumenthal, largely on the basis of Gye's work. The finding by Kauffmann that the power of inducing tumor could be lost by organisms on cultivation, and that of Friedemann, Bendix, Hassel and Magnus that organisms apparently identical with the *B. tumefaciens* of plants, even as regards immunity reactions, but without tumefacient power, could at times be isolated from human inflammatory lesions, supported the idea that some extrabacterial agency was concerned in their action. Bechhold and Smith, indeed, found that the active agent could be filtered through a membrane holding back particles more than 20 micro-microns in diameter, could be boiled for over 20 minutes without destruction, and would resist the action of 1 per cent phenol for 1 hour. But there is no general agreement as to the more significant of these findings. Kauffmann, in his latest work, found that living organisms were necessary for the production of tumor, and regarded the tumors as granulomas. It had been emphasized by Blumenthal, Auler and Meyer that softening or ulceration of animal tumors was necessary for the recovery of the organisms, and Räth reported their rather general occurrence in animal tumors that had been subjected to a process of preliminary artificial digestion. Although his results were regarded as confirmatory by Blumenthal, Räth's later finding, that by this process he could recover Blumenthal's bacillus from the tumors that were caused by its inoculation, seems to cast doubt on the autonomous growth of those lesions, and possibly relegates them to the group of granulomas.

Distribution of Cancer as an Evidence of Infectivity.—Cancer Houses and Localities: There is a considerable body of accumulated literature bearing indirectly on the subject of the infectivity of cancer. In this are a number of instances of local distribution in circumstances such as to suggest the action of a common etiologic factor and hence the possibility of infection. Instances of the localized concentration of cases of cancer are rather numerous; in some cases these are associated with peculiarities of water supply or drainage, while others are without

this association. Among the former are those reported by Alderson in 1885, indicating a riverine distribution; those by Haviland in 1890 and 1895, of "cancer houses" located in regions liable to flooding, although in the later of these reports the possible action of an hereditary factor is mentioned; those by Fiessinger in 1893, who from the usual proximity of such areas to riverine or wooded regions postulated a factor of insect transmissibility; those by Webb, who in 1894 reported on cancer houses that were apparently related to water supply; those by Nason, who in 1898 reported on British registration areas where high incidence of cancer coincided with low-lying, poorly drained land, and those by Blake and by Power, of riverine cancer in England. A summarized report in the *British Medical Journal* for 1900 describes poorly drained regions in Warwickshire, Staffordshire, Shropshire and Worcestershire which had unduly high cancer rates. Mason in 1902 and Plowright in 1904 discussed similar instances, the latter finding especially high cancer death rates in waterlogged areas with surface water in the wells, with 50 per cent of the cases occurring under the 50 foot (15.2 meter) level, and only 10 per cent above the 500 foot (152.4 meter) level. Behla, in the triple community of Luckau, Kalau and Sandau, found frequent cases of cancer in the two former, low-lying settlements, and none in the last, which had a high, well drained site. Pöppelmann reported localized incidence of cancer in a German village with apparent relations to the drainage system. Other reports of similar tenor have been made by Prinzing, Kolb, Unglert, Sachs and Abramowski, the last with an account of a sandy, well drained point that was free from cancer, while on nearby marshy shores it was common; Eichhorn, in a survey of cancer distribution in the district of Constance, in southern Baden, has found this same relation to drainage. Stelys described an apparent case of relation between water supply and cancer, with inferences of causation quite unlike the usual one of infection; in Clermont the disease appeared to show localized distribution along a volcanic fissure of the subsoil, which was the site of numerous carbonated springs, an association which he suggests may be due to the excess of carbon dioxide, deficiency of oxygen or the presence of radio-activity. By no means all the instances of localized cancer concentration are linked with peculiarities of water supply. Wheeler in 1885 reported localized centers in Castine, Me., which had no such relation; Scott in 1900 could find no evidence of such a relationship in eastern Essex; Hoeber in 1904 found that in Augsburg, while cancer was more frequent in the lower portions of the city, these parts were also the oldest and most densely populated. Gordon and Thompson in 1913 found in two registration districts in Cornwall very uneven, patchy distribution of cases of cancer with, however, no apparent relation to water. Nor could Robertson find any suggestion of riverine incidence in the distribution of cancer in

Scotland. There is at least one report of the latter distribution in circumstances in which another, definitely known etiologic factor of cancer plays its part, the riverine occurrence in Kashmir of kangri cancer, as reported by McCulloch.

Other reports of the geographic concentration of cases of cancer without special relation to water supply or to drainage are those of Arnaudet, Lucas-Championniere, Guelliot, Noel, Fabre-Domergue, Loeb (in cattle), Kruse, Sticker, Tynes and Sambon. Some of these have been accompanied by suggestions as to etiology other than that of active transfer of an exciting agent. Loeb believed that the localized occurrence of frequent cases of cancer in cattle from a certain ranch in Wyoming could be best explained by heredity; Kruse ascribed the relatively high frequency of cancer in northern Italy to racial influences, and Pick, writing of the endemic occurrence of thyroid cancer in *Salmonidae*, believed that this was due to some deficiency in their water.

The reports of cancer houses are numerous. Behla, Brand, Guelliot, Fiessinger, Haviland, Lucas, Lyon, Mason, Power, Rohdenburg, Sambon and Webb have reported instances of these, in some cases of unusual interest. Rohdenburg was able in his cases to establish with some regularity, in all the cases of cancer concerned, a common factor of chronic irritation. The usual view of the so-called cancer houses is that of von Hansemann, who regarded them as being purely the result of coincidence. Mathematical analysis is undecided as to this. Pearson, after such analysis of the distribution of cancer in one (British) registration area, believed that it showed the presence of cancer houses beyond the possibility of coincidence, and study of the indiviual houses to his mind excluded explanation by occupation or by heredity. McKendrick, on the other hand, after mathematical analysis concluded that the occurrence of such ho[illegible] was purely fortuitous.

Cancer a Deux: [illegible]rous cases of cancer a deux, such as those collected by Behla, [illegible] Budd, Castueil, Levin, McEwen, Martin and von den Velde[illegible]fficult to interpret in terms of etiology as are the cancer houses. [illegible] unusually suggestive character, as that of Martin, in which a w[illegible]ped a squamous cell carcinoma of the breast at the site where [illegible] husband, to obtain comfort, was accustomed to rest his cancerous ear. While this might be a case of implantation from one person to another, the unusually interesting case of apparent transfer reported by Lecene and Lacassagne does not admit of such an explanation: Here a medical student, in aspirating serous fluid accumulated in a cancerous breast, accidentally injected some of it into his hand, and a few months thereafter an eventually fatal sarcoma developed at the site of the wound. The mathematical probability of cancer a deux has never been satisfactorily determined. Von den Velden believed that his data, based on 1,915 cases of cancer, showed its occurrence in

7 times the probable frequency. Levin, on the other hand, observed cancer a deux in only 18 of 4,000 collected cases.

"Cage Epidemics": Occasional phenomena have been observed in laboratory animals that somewhat resemble those of cancer houses or cancer a deux. Endemic outbreaks of cancers in animals have been described by Borrel, Loeb, Michaelis, Gaylord, Haaland, Negre, Aschner, Henke, Klinger and von Wasielewski. Loeb believed that those reported by him could be best explained by hereditary factors, and while this explanation applies to the outbreak reported by Michaelis, that reported by von Wasielewski was peculiar in that it involved only 1 of 9 cages of similarly bred mice, and Klinger believed that the hereditary element could be definitely excluded in the instance reported by him. Bashford, Murray and Cramer suggested that apparent outbreaks of this sort in laboratory animals might be merely the result of the accumulation of an excessive proportion of aged individuals. In the case of the endemic occurrence of the thyroid tumors of fishes, it has been established by Marine that a large part is played by a deficiency of iodine.

Helminth and Other Higher Animal Parasites.—The association of malignant neoplasms with infestation by various helminth parasites, which was placed on a definite experimental basis by Fibiger in 1913, had been noted clinically for a considerable period before that. Groth in 1864, Klopsch in 1866 and von Linstow in 1868 had reported cases of carcinoma of the breast associated with infection by *Trichina*, and the second named had expressed the surmise that the one process might have a causal relationship to the other. A case of cancer of the breast with trichinosis reported by Strandgaard and one of cancer of the skin by von Langenbeck—references that I have been unable to verify—are cited by Lewin. Since these early reports, cases of this association have been infrequent, probably because of the more effective control of the parasite, although Babes reported a case of bronchial carcinoma, and Babler one of cancer of the lip, associated with trichinosis, and in 1920 Fibiger was able to cite another case, of cancer of the tongue.

Schistosoma haematobium as a tumefacient agent was first reported in connection with polyps of the bladder by Sonzino in 1876 and by Couenon in 1881. Belleli in 1885 observed what he regarded as this parasite—more probably it was *S. mansoni*—in association with a rectal fibro-adenoma, and in that year Kartulis reported that the tumor-like growths of the bladder were of such a character as to lead occasionally to rupture. Apparently, however, the first definitely diagnosed carcinomas associated with *S. haematobium* were those reported by Harrison in 1889. Albarran and Bernard in 1897 reported a similar case, and in 1898 Kartulis reported not only a number of cases of vesical cancer, but also one of cancer of the foot which showed relations to the parasite,

and occasional cancers of the rectum and prostate (*S. mansoni?*). The most comprehensive discussion of the relationship of *S. haematobium* to cancer is that of Goebel, who called attention to the stage of preliminary irritation induced by the parasite, as well as to the blockage of blood and lymph vessels by the ova.

In 1890 Toison reported the finding of unidentified ova in the central portion of a sarcoma, and in 1898 Kanamori described in detail the observations in a case of rectal carcinoma associated with the ova of a parasite unidentified at that time, but since recognized as *S. japonicum*. That this case was not exceptional was shown by Kazama, who in 1921 pointed out the frequency of intestinal cancer in districts infested with that parasite, and its association with the ova. Obviously tumefacient properties would appear to be generally associated with *Schistosomidae*.

Distomidae would appear to have almost equally great powers of inducing tumor. Askanazy in 1900 reported in East Prussia the occurrence of hepatic cancer associated with *D. felineum*, and Katsurada 2 cases of cancer of the liver among 76 cases of infestation with *D. spathulatum* studied by him. In addition to its occurrence in East Prussia, *D. felineum* has been reported as causing cancer in Russia, by Ruditzky, and in Holland, where Hoogland found 5 cases of cancer of the liver in infested cats and reported on the apparent association of *Distoma* with cancer of the liver in cattle. With this parasite, as with *S. japonicum*, the preliminary changes are those of chronic inflammation; Paul in 1927 called attention to its action in inducing cirrhotic changes in the liver.

A rather wide range of parasites have shown relationships to cancer in lower animals, in addition to those already cited in relation to human cancer. Borrel in 1906 reported on the frequency of rat sarcomas in connection with *Cysticercus*, a finding confirmed by Regaud in 1907, Bridre and Conseil and also McCoy in 1909, Woolley and Wherry in 1911 and 1912 and Bullock and Rohdenburg in 1913. Experiments at the induction of tumor with this parasite, *Taenia crassicaudes*, were made with very doubtful success by Saul in 1907, but in 1920 Bullock, Curtis and Rohdenburg were able to produce sarcomas of the liver in rats fed with the eggs of the parasite. Here, too, as they had shown in a previous report, the development of a malignant condition is preceded by changes of a chronic inflammatory character. Of miscellaneous parasites that have from time to time been connected with the appearance of tumors in animals are: *Trichodes crassicauda*, which Loewenstein reported in 1910 as causing papillary tumors of the urinary bladder in rats, occasionally with some indications of malignancy; even proximity of the ova appeared to suffice to induce the changes, which were without evidences of preceding inflammatory hyperplasia; *Rhabditis pellio*, reported in 1919 by Kopsch as causing both carcinomas and sarcomas in infested

frogs; experimental attempts at the induction of cancer by this parasite, reported by Puder in 1925, were unsuccessful. Other parasites are *Dispharagus sp.*, which von Wasielewski and Wülker found associated with malignant tumors in the stomach of the dove; *Hepaticola hepatica*, which Beatti in 1923 found in connection with gastric cancer of the rat, a finding that was confirmed by Bonne in 1926 and studied experimentally by Vogel in 1929 with reported success, although the photomicrographic illustrations that accompany the report are not entirely convincing; *H. cancerogena*, a parasite regarded by Beatti as distinct from *H. hepatica*, which he found also frequently associated with gastrointestinal cancer in the rat, although feeding experiments on pied rats were unsuccessful. A number of interrelationships between parasites and tumors are less definitely established. This is particularly true of the suggested connection between acarid infestation and cancer. Borrel, who in 1906 suggested that parasites might be the carriers of a cancer virus, in 1908 and 1909 reported the frequent presence of these parasites in human facial cancers; Negre in 1910 ascribed a cage epidemic in mice to the agency of these parasites, and Saul in that year called attention to an allegedly wide distribution of acarids in human and other tumors. Among the acarids found by Saul was one identified by Dahl as a member of the genus *Tarsonemi*, acarids that in plants evoke gall formation. Ascher, also in 1910, observed in rats an acarid infestation that caused papillary proliferation and limited downward growth of the skin. Von Wasielewski, too, in 1912 found limited epithelial overgrowth in rats irritated by these parasites, and Chambers and Somerset in England in 1923 found acarid infestation frequently associated with cancer of the breast. In 1928 Sambon suggested an association might be established between cancer of the skin in mice and the presence of *Psoergetes simplex*. Du Bois, cited by Lewin, reported a case of cancer of the skin apparently having its inception at the point of lodgement of an acarid.

On the other hand, Tsunoda in 1910 found the same relative frequency of acarid infestation in normal and cancerous breasts, and Orth objected to their implication as causative agents of cancer on several grounds—the data of Tsunoda, the lack of relationship between the age incidence of the two conditions, and the fact that these parasites are especially frequent in hair follicles, which rarely become cancerous. Reuter published observations showing the readiness with which acarids could invade tissues after their removal from the body. Joest and Ernesti in 1916 could find no relationship between acarid infestation and cancer in birds, and Marsh, although he admitted the possibility of their acting as chronic irritants, found them only rarely in tumors in the mouse, and could find no experimental evidence of any relationship to cancer. Similarly Teutschlaender in 1919, although he found

that these parasites in rats caused a form of itch, could find no relationship of this to cancer in that animal. However, in 1922 he reported a cancer of the foot in a chicken locally infested by acarids. Amormino in 1929 found in rabbits that acarids caused only cutaneous inflammation.

Other parasites of somewhat doubtful association with cancer are the filariae, which were reported by Borrel in 1927 as being of frequent occurrence in the spontaneous cancers of the breast in mice, a finding that is disputed by Dobrovokskaia-Zavadskaia and Kobozieff. *Echinococcus* infestation has been suggested as an occasional cause of cancer of the liver by Ziegler. Naück and Liang reported two carcinomas of the bile tracts in the liver, in association with the presence there of *Clonorchis sinensis.*

In spite of the frequently observed association between various parasites and cancer, this association might still be regarded as coincidental if it were not for the work of Fibiger, to which allusion has been made. Basing his work on the observation of frequent gastric cancers in rats from the warehouse district of Copenhagen, he was able to establish the fact that these were related to infestation with a parasite named by him *Spiroptera neoplastica,* and the further fact that the intermediate host of this parasite was a roach of American origin, evidently transported to the warehouses by ships. Feeding experiments with these roaches definitely showed their relation and that of the parasite to the rat cancers, and by means of them he was able to produce cancers of the stomach not only in the pied rats in which most of his experiments were conducted, but, though less frequently, in other strains of tame rats as well, and, though rarely, in mice. With the exception of the causation of malignant tumors by Marie, Clunet and Raudel-Lapointe by means of the x-ray, which was essentially the repetition in experimental conditions of a well established clinical fact, this represents the first experimental induction of cancer strictly de novo.

As to the mode of action of the animal parasites in inducing malignant growth, little is known. Their mere ability to induce chronic inflammatory changes, although, as has been shown, frequently associated with the appearance of cancer, cannot alone suffice to explain their tumefacient effects. A number of irritants that produce inflammatory changes of equal or greater degree are only exceptionally associated with the causation of cancer, as was pointed out by Fibiger in connection with infestation by *Trichodes,* a parasite of the same size, and producing direct effects similar to those of *Spiroptera,* but rarely if ever causing cancer. Borrel in a number of publications views these parasites as the carriers of a presumably living virus, and Mercier and Lebailly have suggested that a myxosarcoma of the fowl which they reported may have been caused by the transfer by acarids of a filtrable

agent of the Rous type. Saul in 1915 ascribed their action as due to metabolic products, an explanation which, as far as it goes, seems as satisfactory as any that is likely to be adduced. The finding of Flury and Leeb that the metabolism of *Distoma* is largely of anoxybiotic type, is interesting in view of the experiments on tumor metabolism of Warburg, and hints toward a possible clue in that direction.

The Filtrable Agent.—The discovery by Rous in 1911 of the first of a series of fowl tumors that are transferable by cell-free material introduced into the study of the etiology of tumor an element that even yet is not satisfactorily explained. Quickly following the report on this first tumor, the finding of a number of similar ones was announced by Rous, Rous and Lange, Tytler, Fujinami, Muto, Inamoto and Ogata and Ishabashi and later cases by Begg and Teutschlaender. These tumors are all alike in that they occur in fowls, and in that they may be transmitted by almost certainly cell-free filtrates, by properly prepared desiccated tumor tissue and by glycerinated extracts. They vary in morphology, but the morphologic type of the original tumor is as a rule preserved in the induced tumors, although to a certain extent, as shown by Rous and Murphy, variations in the tumor may depend on the host. In addition to the confirmation of Rous' findings as afforded by the discovery of similar tumors, his detailed observations were quickly confirmed by Walter and Bürger, and since by an enormous volume of related work. Bürger found that not only could these tumors be transmitted by the means mentioned, but also at times by the blood or by the ascitic fluid of involved animals, and even by tumor-free organs. As regards transmission by blood, Llambias and Brachetto-Brian were able to show that not only whole blood, but even washed red cells, could be effective, and Lewis and Andervont, that this was true both of plasma and washed leukocytes, the latter being the more certain. Fraenkel found that while splenic tissue, free from metastases, could cause these tumors at times when inoculation with blood gave negative results, no results could be anticipated from either unless the tumor had progressed to a considerable size, and that then even the yolk of an ovarian egg might be active.

In addition to the specificity as to type of tumor, these filtrates also show marked specificity as to animal susceptibility. With the original Rous tumor, this was at first limited to related fowls, and only later did Rous and Murphy find that the selectivity gradually disappeared, and while eventually they found that the virus was capable of inducing tumor in embryonic pigeons and ducks, this is restricted, as with the transplantation of tissues, to the earlier nonspecific stages of development.

Closely related to the action of the filtrate is the matter of injury to tissue, as was early shown by Rous and his co-workers, who found that care taken to minimize this greatly reduced the frequency of the induction of tumor. That this is possibly due to fixation of the agent by

injured or regenerating cells is suggested by the work of Pentimalli, who found that injured cells and embryonic cells possess such power of fixation, and Mackenzie and Sturm, who found the virus localized in the early stages of induced local inflammatory reactions. In the opinion of Rous, Murphy and Tytler, the metastases that are a prominent feature of these tumors seem largely to be the results of cell transplantations, as with the general run of malignant tumors, except to the extent that they might result from the action of the agent on injured tissues; however, Fischer has found that in tuberculous fowls the tubercle bacilli may be found in the primary tumor only, which would apparently indicate the induction of metastases by the cell-free agent.

As to the nature of this agent, in many respects it obviously resembles an ultramicroscopic living virus, even to the presence in the tumor cells, as shown by Borrel, of granulations like those of molluscum or vaccinia. Such was the view taken by Gye, who in 1925 published the results of experiments that appeared to indicate that it contained such a virus, capable of cultivation, which could be destroyed by the action of certain antiseptics, whereupon the filtrate became inactive until its activity was restored by the addition of other tumor extracts, even from entirely unrelated animals. In other words, the fresh filtrate of the Rous tumor contained two elements necessary for the induction of tumors—a living, nonspecific virus and a specific adjuvant, the presence of which permitted the attack of the virus, and which gave the filtrate its properties of specificity both as to host and as to type of tumor. Gye's work offered by far the most satisfying explanation of the properties of the filtrable agent and received a certain amount of confirmation by Baker and in part by Mackenzie and Illingworth and Sittenfield and Johnson. But by far the greater number of experimenters who checked Gye's work were unable to find confirmatory evidence. Even in the year of Gye's original report, Roussy expressed the doubt that chloroform, the first antiseptic used by Gye, could be relied on to destroy with certainty the supposed virus, and Cutler in 1926 and Fraenkel in 1926 and 1927 published experimental results indicating that such was the case. It was quickly shown, too, by Flu, Murphy, Sternberg and Cori, that various non-neoplastic tissues—placenta, embryonic tissue, cultures of normal organs, even foreign blood—could supply what Gye regarded as the nonspecific virus. Pentimalli in 1927 repeated Gye's work with careful observance of his technic, without being able to confirm his results, and a repetition of Gye's work undertaken by Gye and Mueller, with the use of acriflavine as an inactivator, gave results so uncertain as to be on the whole nonconfirmatory. And Kolmer and Harkins, using concentrations of chloroform and acriflavine in degree sufficient to insure inactivation, were unable to find any evidence of a restoration of tumor-producing power. Illingworth and Alexander, too, found that

inactivation by ultraviolet light did not admit of reactivation. With experiments as complicated as those of Gye, the possibilities of uncertainty are numerous. The unreliability of dilute antiseptic agencies has been mentioned. Murphy explained the positive results as due to the presence of a ferment that, after inactivation, was still capable of reactivation; Harkins, Schamberg and Kolmer believed that the alleged specific factor represented a still active, although attenuated, filtrable agent; Haaland, repeating Gye's work on the causation of mouse cancer by apparently cell-free cultures, found that the supernatant broth if entirely cell-free was incapable of producing tumors, while the cell-containing sediment could produce tumors by inoculation; in this respect, he is not in agreement with Sittenfield and Johnson, who obtained a very considerable percentage of successful inductions of tumors, by the use of rabbit serum broth cultures of mouse sarcoma, even though these had been originally inoculated with the Berkefeld filtrate of the tumor—results like those of Gye with similar tumors. Simon and Beck, who worked with an artificially induced tumor of the Rous type, believed that the occasional positive results observed in Gye's procedures were to be explained by a summation of subinfective doses or by activation of a subinfective dose; and Mueller, who in 1927 was unable to confirm not only the work of Gye, but that of Murphy and of Flu as well, believed that there were so many uncontrollable local and individual factors as to make uncertain any results that might be obtained.

A number of rather indirect efforts have been made to determine the nature of the agent. Some of these would suggest that even with the filtrate the causation of tumor is a matter of cellular transfer. Nakahara, who found that the action of the filtrate is greatly impaired by alternate freezing and thawing, and by acetone—behavior strikingly unlike that of known ferments—and who found that prolonged and excessive pulverization of the desiccated tumor would destroy its action—believed that this is due to the presence of filter-passing, minute cells, which he believed he could observe in the filtrates. This view has not been generally accepted, and in regard to the pulverization experiments, it may be pointed out that such a process would greatly enhance oxidation, which from other evidence is known to destroy rapidly the agent in question. Fraenkel believed that the possibility of cellular transmission could be absolutely excluded, basing his opinion on quantitative experiments, on the immunity of the agent to various physical and chemical agents and on the correspondence in the behavior of the agent thermally, chemically and biologically to that of the ferments. Investigation of the particulate size of the agent, which might be hoped to throw some light on the matter of possible cell transplantation, has yielded rather conflicting results. Rous and Murphy believed that its size, as determined by filtration experiments, would indicate an organized structure even to

the point of microscopic visibility; Jung found in an active de Haan filtrate formed elements that suggested the possibility of implantation; Ono found that the agent could pass through a Zsigmondy-Bachmann filter and through a Bechhold collodion membrane with a probable pore diameter of 0.6 micron, and Fraenkel, through a filter of a pore diameter of 10 micromicrons; Zinsser and Tang, using filtration through aceto-collodion membranes, found for the virus a size greater than that of casein particles, smaller than that of arsenious sulphide, and comparable with that of bacteriophage or herpes virus; Baker and Peacock, who found that the agent was less susceptible to the action of ultraviolet light than are common bacteria, believed that this was due to its smaller particulate size.

Although the action of the agent is on the whole suggestive of that of the filtrable living viruses, this similarity does not hold on detailed comparison. Loewenthal found a decided difference in this respect in comparing the respective responses to capillary attraction. As concerns its powers of adsorption, the testimony is conflicting; Fraenkel, Mislowitzer and Simke found that while it was adsorbed by casein, kieselguhr and kaolin, it was not taken up by animal charcoal, and in general that it showed some similarity in these respects to the filtrable viruses; Lewis and Andervont, on the other hand, failed to obtain its adsorption by kieselguhr, kaolin, india ink or charcoal, and so found a decided difference from the behavior of the viruses of vaccinia and fowlpox. A peculiarity of its behavior found by Lewis and Lewis and Andervont was that it was inactivated by a number of aluminium salts, the acetate proving to be an exception to this rather general effect; also by certain calcium salts, the composition of these being such as to suggest a negative charge for the agent—a conclusion confirmed by Murphy's and Pulcher's experiments with cataphoresis and by Pulcher's experiments on its adsorption by oxyhemoglobin. In this respect the agent probably resembles the known filtrable viruses, as that of vaccinia was shown by Douglas and Smith to have a negative charge, although Natajaran and Hyde were unable to effect any separation by the cataphoresis of a number of these viruses.

While none of the data just cited are particularly decisive, striking evidence against the (living) virus character of the agent lies, as was pointed out by Murphy, in its extreme specificity; to postulate such a virus as the cause of the several tumors of the Rous type would necessitate the conception of a separate virus for each, since each is highly individual in the matter of predilection for a host, and particularly in the matter of the type of tumor that it produces.

The most conclusive evidence, however, against the theory of a living virus lies in the several reports of the causation of this type of tumor by purely artificial means. Carrel and his co-workers produced

a number of such tumors by the insertion into fowls of embryonic chick tissue, along with the injection, not necessarily at the same site, of such substances as dilute arsenious acid, indol and tar. While not nearly all such attempts have been successful—Deelman was unable to obtain Carrel's results with arsenic, and Sturm and Murphy, while they obtained a sarcoma by the injection of tar and embryonic tissue, failed to get one transmissible by filtrate—the attempts have been successful in enough cases to establish this as one method of tumor production. White and Brebner secured directly confirmatory results. To the extent that such tumors are like the spontaneous Rous tumors transmissible by filtrate, they open two possibilities as to the character of the filtrable agent: Either it is a living virus, in which case it is necessary to assume that it is harbored by a considerable proportion of normal fowls and remains latent in them throughout their lives, or it is a metabolic product which has the somewhat unusual character of being formed by the lesion that it induces. While the experiments of Carrel leave the question undecided, later experiments by Fischer, Laser and Haagen would seem to indicate the greater probability of the latter hypothesis. Fischer, and after him Haagen, reported that cultures of chick monocytes in vitro which were treated for a number of generations with dilute arsenic pentoxide gradually assumed the cultural characteristics of malignant cells, and on inoculation into adult fowls set up in them rapidly growing sarcomas, like the Rous tumors in respect to transmission by filtrate. Laser, using the plasma of a fowl that had been subjected to injections of coal tar and cultured monocytes, similarly produced a Rous sarcoma artificially. With these experiments, too, the results are not constant, as was shown by DeFries, who was unable to repeat Laser's work successfully. From the fact, however, that malignancy of the Rous type may be induced in tissue cells in vitro, in circumstances that would apparently absolutely preclude the possibility of infection, it would seem that the filtrable agent would almost certainly be of metabolic origin.

The third possibility as to the character of the agent is that it is of the nature of a ferment or enzyme. It has already been stated that Fraenkel, who found that it was separable by the methods for the isolation of ferment, considered that enough similarities exist to place the agent in this category, while Nakahara took the opposite view. Haagen, discussing the affinity of the agent for monocytes, expressed belief that it is a ferment acting largely through reduction of surface tension; Duran-Reynals and Murphy found that it could be fixed by the muscle tissue of fowls, but not by that of other animals, a behavior suggestive of that of ferments; its adsorption by injured or regenerating tissues would also be compatible with that view. Lewis and Michaelis found that the range of hydrogen ion concentration that permits action of the

agent is similar to that of bacteria, being less restricted than that of animal cells, and is compatible with that of ferments. That the agent is associated with certain protein constituents has been shown by Sugiura and Benedict, who found it in the globulin fraction; by Fraenkel, who would place it in the euglobulins, and by Murphy, who, after concentration by cataphoresis about the anode, obtained a nucleoproteid that appeared to be the agent itself. It shows a susceptibility to oxidation that could well be associated with the character of a ferment, as was shown by Murphy and by Fraenkel. Mueller showed that this oxidation could be prevented by the addition of dilute cysteine hydrochloride.

There are some, although much less definite, indications that an agency similar to that of the Rous tumor, at least in respect to filtrability, may play some part in animal tumors. A number of allegedly successful attempts at the induction of tumors by means of cancerous fluids, or cultures of such fluids, have been cited previously, but most of these have been of rather doubtful authenticity. Of more recent workers, Gye based part of his theory on his success in producing tumors in mice by the inoculation of rabbit serum potassium chloride broth cultures of mouse sarcoma, after anaerobic incubation. Sittenfield and Johnson's corroboration of these results was somewhat less successful than the work of Gye, but they were able to report the induction of 34 tumors in 105 attempts, while with the Berkefeld filtrate of the cultures they obtained only 6 tumors in 164 attempts. That the positive results could not be ascribed to implantation of surviving cells they believed could be shown by the small number of tumors that followed the introduction of the tissue itself after the same period of incubation. Using mouse carcinoma tissue, however, in similar experiments, a much smaller yield of tumors was obtained—only in 2 of 81 attempts. As already stated, similar experiments by Haaland were completely unsuccessful; Auler and Blumenthal, on the other hand, apparently succeeded in causing tumors in rats by the use of edematous fluid from human cancers, either with this alone or in combination with the cell-free filtrate of lymph glands from cancerous rats—Auler with a total of 3 successes in 16 attempts, Blumenthal with 3 in 36 attempts. In regard to these experiments, those of Sittenfield and Johnson showed a marked distinction between the results obtained with the unfiltered, and those with the filtered, culture fluids. Dealing more specifically with cell-free filtrates, a number of earlier attempts at the causation of tumors by these were completely unsuccessful, as reported by Herzog, Henke and Schwarz, Koenigsfeld and Prausnitz, Bayon, Romme and Harde, Koch, Pearce and Murphy, Harde and Henri, with tumors of rats, mice or rabbits. Morris in 3,000 mice into which the filtrates had been injected observed tumor development in only 4 of a limited number of survivors, and these in splenectomized animals in which the injection had been

accompanied by that of kieselguhr. Gaylord and Simpson, on the other hand, reported a small number of rather doubtful successes in the case of rat tumors. Rather recent work, however, of Burrows, Bisceglie and Erdmann affords evidence that such filtrates may play a part in the induction of mammalian tumors. Burrows, working with rats, found that in a small proportion of experimental animals in which had been implanted embryo tissue of the same descent, after immersion of this in the filtrate of the Jensen rat sarcoma, there was a late development of sarcoma at the site of implantation. A much greater number showed limited proliferation. Bisceglie, working with chick embryonic tissue, which was introduced into mature fowls along with the filtrate of mouse carcinoma, likewise found that this became sarcomatous, although similar attempts in mice gave negative results; in another series of experiments, it was found that if tumor filtrates were injected before the transplantation of neoplastic tissue, the malignancy of the latter was greatly enhanced. Erdmann found it possible to produce tumors of the Flexner-Jobling type in rats in which she injected a filtrate of that tumor after blockage of the reticulo-endothelial system by india ink; a few of her animals—all were of a strain very susceptible to the tumor experimented with—developed sarcomas from the filtrate alone. But results such as these, although apparently well established, are on the whole exceptional. Shattock and Dudgeon in 1916 reported that adult or fetal tissues of the mouse, implanted in adult animals after immersion in cell-free tumor extracts, failed to show tumor growth. Rous, experimenting with mixed grafts of tumor and embryonic tissue, failed to find any evidence of impartation of malignant growth to the latter. Certainly the action of these mammalian filtrates is in little degree comparable to that of the Rous type of fowl tumors, and their action appears to be only an occasional and exceptional feature of mammalian tumors. A much more constant effect is that of a temporary stimulation of growth; the limited proliferation observed by Burrows in many of his animals has been noted; Drew found that tumor extracts, made during periods of rapid tumor growth, stimulate active proliferation of cells in vitro—an effect that is not shown by extracts of normal organs except after autolysis; Eggers, studying the action of filtrates from a rapidly growing rat sarcoma on independently induced connective tissue growth, found with great regularity an enhancement of this, but one of temporary duration. There is some evidence that this property is common to all growing tissues, as Carnot and Terris found that material derived from fetal or regenerating tissues accelerates regeneration.

One report by Kritschewski and Rubinstein stands in apparent contradiction to the general findings just described. It deals with an alleged sarcoma of the rat, developed in response to the injection of human melanosarcomatous tissue, and transmissible by filtrate. The

findings are so exceptional in several respects, and the photomicrographs are so unconvincing as to make their identification of the lesion as neoplastic very doubtful.

Carcinoma Sarcomatodes: The cases of carcinoma sarcomatodes, in which there appears to be a transfer of malignancy from one cell type to another, or the occurrence of two types of malignant growth simultaneously, would suggest the action of some extracellular agent by which that transfer is effected. Quite a number of human cases of this sort have been reported—by Schlagenhaufer, Herxheimer, Taylor and Teacher (6 cases), Reichmann, Lippmann, Lindemann, Leuenberger, Schiller and Schmorl (cited by Apolant), and while in some cases, as in that of Rothacker, the two malignant growths would appear to have originated independently, in many the evidence would at least suggest a transmission of malignant characteristics from one type of cell to another. There are also fairly frequent instances of similar changes in animal tumors, some observed in experimental circumstances. A number of mouse or rat tumors, originally carcinomatous, in later generations becoming sarcomatous, were reported by Ehrlich, Apolant and Haaland, Loeb, Liepmann, Haaland, Stahr, Russell, Woglom, Bashford, and Asada and Okabe. An exceptional case of this sort was reported by Lewin, in which a rat adenocarcinoma in later generations developed into a squamous cell carcinoma, a spindle cell sarcoma and a round cell sarcoma. Most usually the change is from carcinoma to sarcoma, but the reverse phenomenon has occasionally been observed—by Coenen with human material, by Lewin in rats, by Schöne in a dog, by Duschl, who observed a fibro-adenoma develop at the site of an apparently unsuccessful transplantation of rat sarcoma, and less certainly by Flexner and Jobling, who in a rat tumor originally almost wholly sarcomatous observed a later change to adenocarcinoma; but careful study of the original tumor revealed a mixed character there. Nicholson observed the development of a small epithelial tumor, apparently cancerous, in the wall of a sinus leading to an inoculated sarcoma.

It is possible that with some of these tumors the change may be more apparent than real. Asada and Okabe, in the case of the mouse tumor referred to in the foregoing paragraph, in which there was this apparent transfer of malignancy from epithelial to connective tissue, believed that the change was really only one of appearance in the epithelial cells, and Wail reported similar observations. But that this is not universally true is indicated not only by the careful morphologic studies of some of the experimental tumors of this type, but as well by such cases as that studied by Lewin, in which there is a series of changes too great to be reasonably explained by variation in cell form alone.

Comment.—Viewing the question of parasitism as a possible cause of cancer from the intrinsic evidence alone, without reference to the

relations of malignancy to chronic irritation, it would seem to be rather well established that all the work done in the search for a specifically causative parasite has been largely a chase after the will-o-the-wisp. None of the alleged parasites reported so hopefully has withstood the test of critical reinvestigation. It is equally well established, however, that parasitism, albeit nonspecific, may cause cancer, as with the several cancer-causing helminths. To what extent their action in inducing malignant growth is to be sought in their effects as chronic irritants, to what extent their action is even more obscure, are questions to which there is as yet no answer.

There can be little doubt that there is an agency, capable of release from the cell, which in suitable conditions is capable of inciting malignant growth. Definitely present in a number of fowl tumors, it has been studied there in some detail, although as yet the knowledge of its exact character is far from clear. The preponderating evidence would seem to indicate that it is not an independent form of life, nor in that sense a living organism; but to view it as a product of cellular metabolism, which can in turn excite the metabolic reactions that give rise to it, does little to enlighten one as to what it really is. Some hints there are that a somewhat similar agent may play a part in at least some mammalian tumors and possibly in the tumefacient bacteria; but here even the evidence of the existence of such an agent is relatively slight, and to what extent it will be found similar to the Rous type of virus can only be conjectured.

In spite of the evidence that parasites of one kind or another may cause cancer, there is little to indicate that such parasitism plays a large part in the causation of human tumors. The helminth and other gross animal parasites only occasionally play a rôle here. In regard to the tumefacient bacteria, one cannot speak so confidently. But that those organisms are to be looked at as common carriers of malignancy from one individual to another would appear to be almost entirely impossible; even their discoverers found them in less than half of the tumors they examined; their power to induce malignant growth appears to be capricious; and in spite of the numerous instances in medical literature of apparent transmission of cancer from one individual to another, those instances are the exceptions rather than the rule, and are most certainly contrary to the usual facts.

University of Nebraska College of Medicine.

Notes and News

University News, Appointments, Promotions, Resignations, etc.—John Glaister, Jr., professor of forensic medicine in the University of Egypt, has been appointed to the chair of forensic medicine in the University of Glasgow, now occupied by his father.

Sydney C. Dolrimple has been appointed instructor in pathology in Tufts College, Boston.

Dudley A. Robnett has resigned as assistant professor of pathology in the University of Missouri.

Clarence G. Pflaum, instructor in pathology in the University of Minnesota, has been appointed assistant professor of pathology in the University of Missouri.

In the University of Pennsylvania Stuart Mudd has been promoted to associate professor of bacteriology.

Slikh Poloyes has been promoted to assistant professor in pathology in the Long Island College of Medicine.

The twenty-fifth anniversary of Walter B. Cannon's professorship of physiology in Harvard University was celebrated on October 15 by public exercises at which a portrait of Dr. Cannon was presented to the school.

Howard T. Karsner, professor of pathology in Western Reserve University, succeeds the late Aldred S. Warthin as member of the council on physical therapy of the American Medical Association.

In the University of Texas Ellen D. Furey has been appointed adjutant professor of pathology in place of Harry L. Klotz, and John F. Hilcher has been appointed instructor in pathology.

In the Woman's College of Philadelphia S. Brandt Rose has been appointed professor of bacteriology.

It is reported that Otto Warburg of the Kaiser Wilhelm Institute for Biology in Berlin has been awarded the Nobel Prize in medicine and physiology for 1931 for his work on the mechanism of oxidation in the living cell.

The National Research Council has given Paul E. Steiner a medical fellowship for work in pathology.

Society News.—The Society of American Bacteriologists will hold its annual meeting in Baltimore, Dec. 28 to 30, 1931. In the general scientific session a series of papers will be presented on "Bacterial Dissociation and Life Cycles." The topics for discussion in the section on medical bacteriology and immunology include hemolytic streptococci, meningococcus and the immunologic aspects of epidemic poliomyelitis and typhus fever.

Commemoration of Discovery of Tubercle Bacillus.—The fiftieth anniversary of the discovery of the tubercle bacillus by Robert Koch, March 24, 1882, will be commemorated by suitable ceremonies in Berlin from May 17 to 20, 1932.

Medical Fellowship Board, National Research Council, Meets in March.—The next meeting of the medical fellowship board of the National Research Council will be held in the latter part of March, 1932, and applications to receive consideration at that time should be filed not later than February 15 next.

The Biological Photographic Association.—An organization by this name has been formed to promote the use of photography in its various forms as aid to biologic research and medical observation and record. The president is Ralph P. Creer, director of photography in the school of medicine of Yale University, and the secretary is Theodore Nelcey of the same school.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

EXPERIMENTAL NEPHROTIC EDEMA. LOUIS LEITER, Arch. Int. Med. **48**:1, 1931.

Edema has been regularly produced in dogs by adequate plasmapheresis and the resulting reduction in the plasma proteins. The critical level of edema of the plasma proteins is about 3 per cent. An interruption of the depletion of the plasma is followed by an immediate increase in the plasma proteins and an equally rapid disappearance of the edema by diuresis. The disturbance in the water balance seems to be largely a temporary upset in the normal equilibrium between the capillary hydrostatic or filtration forces and the plasma protein osmotic or reabsorption forces. It is not primarily dependent on cardiac, renal or capillary permeability factors. This experimental edema is nephrotic, as demonstrated, for the first time, by actual quantitative analysis of the protein content of the transudates. The low protein content lies exactly within the range of that of the edema fluids of clinical nephrosis and is essentially of the same order of magnitude as the concentration of protein in normal cerebrospinal fluid and of that in other ultrafiltrates of the plasma. The study of the regeneration of the plasma proteins in the depleted dogs has confirmed the earlier work of Whipple and his associates, which showed that globulin was reformed faster than albumin. The blood cholesterol tended to decrease during vigorous plasmapheresis. With cessation of bleeding, the cholesterol usually rose, occasionally to values considerably above normal. This was, however, only a temporary phenomenon and was in no way suggestive of the hypercholesteremia of the nephrotic syndrome in man. Histologic study of the kidneys in these experiments has failed to produce any clearcut evidence to support the contention of Barker and Kirk that hypoproteinemia leads to contracted kidneys. In fact, the observations in the control experiments and other facts reported seem to lead directly to the conclusion that Barker and Kirk have erred in two directions: First, they have overlooked the possibility and the frequency of "spontaneous" contracted kidneys in dogs. Second, they have apparently failed to realize the lack of essential resemblance between the so-called renal lesions of plasmapheresis and the histologic changes in the kidneys of patients with so-called nephrosis. In view of this confusion, it becomes evident that experimental nephrotic edema in dogs is not quite the same as "experimental nephrosis." Experimental nephrosis has not yet been produced. The rôle of starvation, of the administration of salt and water by stomach tube and of certain other factors in the experimental procedure has been controlled to a certain degree. The relation between experimental nephrotic edema and other experimental and clinical edemas has been discussed. In collaboration with Dr. I. S. Falk, we have presented the first published data on the protein content of the plasma and of the edema fluid in a "spontaneous" nephrotic edema occurring in a monkey.

AUTHOR'S SUMMARY.

NEPHROSCLEROSIS. PHILLIP F. SHAPIRO, Arch. Int. Med. **48**:199, 1931.

There is no fundamental difference in the pathogenesis of benign and malignant nephrosclerosis, whatever their respective etiologies may be. Both depend, however, not on glomerular ischemia or on arteriolar occlusion, but on a hyperemia associated with a retardation of flow. This conclusion was suggested by a histologic study, checked by a series of experimental injections. The retardation is based on a neurogenic dissociation in reaction between arterial constriction and peripheral dilatation. The changes that follow are analyzed on the basis of

Ricker's views on renal hemodynamics. Moderate retardation leads slowly to the organic changes of benign nephrosclerosis. Severe retardation rapidly induces the pathologic changes of malignant nephrosclerosis and terminates in uremia. Malignant nephrosclerosis is therefore accepted simply as an "atherosclerosis renum accelerata gravis."

AUTHOR'S SUMMARY.

THE RÔLE OF THE PYLORIC SPHINCTER IN THE CONTROL OF GASTRIC ACIDITY. R. ELMAN and A. P. ROWLETTE, Arch. Surg. **22**:426, 1931.

When the pyloric sphincter is sectioned, a fall in gastric acidity follows. There is also an increase in emptying time. This would indicate that the pylorus exercises control over gastric acidity by controlling the reflux.

N. ENZER.

GALLBLADDER FUNCTION. I. S. RAVDIN and J. L. MORRISON, Arch. Surg. **22**:810, 1931.

The authors detected rhythmic contractions of the gallbladder in the dog, guinea-pig and monkey. On one occasion, contractions of the human gallbladder were noted. Some of the constituents of bile are found in the pericholecystic lymphatics, but not all of the constituents. The conclusion is that concentrated bile leaves the gallbladder by the cystic duct.

N. ENZER.

THE EFFECT OF SUPRANORMAL TEMPERATURES ON TISSUE CULTURES. G. PINCUS and A. FISCHER, J. Exper. Med. **54**:323, 1931.

In a study of the growth of chicken osteoblasts in cultures exposed to supranormal temperatures of 42, 44, 47, 50 and 52 C., it has been observed that there is no lethal effect after prolonged exposures at 42 and 44 C. Cultures are killed after an exposure of 105 minutes at 47 C., of 6 minutes at 50 C. and of 3.5 minutes at 52 C. A definite inhibition of growth occurs after different exposures at all temperatures from 44 C. onward. There is a latent period of approximately 24 hours before any effect of sublethal or just lethal exposures becomes discernible; this latent period appears to be independent of the duration of the sublethal exposure and of the temperature. The high temperature coefficients for lethal exposures and for exposures just sufficient to inhibit growth indicate an underlying "destructive" process in the cells of the culture.

AUTHORS' SUMMARY.

IS THE RAT DERMATITIS CONSEQUENT ON VITAMIN B_2(G) DEFICIENCY TRUE PELLAGRA? S. S. GURIN and W. H. EDDY, J. Exper. Med. **54**:421, 1931.

Isolated cases of dermatitis resembling histologically that of human pellagra have occurred in rats supplied with sufficient vitamin B_2(G) in the form of beef extract or neutral autoclaved yeast to produce good growth. In other rats on basal diets containing similarly prepared nutrients, but deprived of any known source of vitamin B_2(G), a dermatitis develops similar in appearance to that described by other workers, but this skin effect differs in the histologic picture from that found in human pellagra or that found in black tongue of dogs. These rats showed failure of growth, which supports the view that they lacked growth-promoting vitamin B_2(G). It is suggested that dermatitis in rats may be of diverse types: one resulting from vitamin B_2(G) deficiency and quite different histologically from human pellagra, and one closely allied to human pellagra and black tongue of dogs due to lack of some at present unidentified factor.

AUTHORS' SUMMARY.

THE LIPOLYTIC ACTIVITY OF TISSUES OF TUBERCULOUS GUINEA-PIGS. F. C. HAPPOLD and A. TAYLOR. Brit. J. Exper. Path. **12**:272, 1931.

The lipolytic activity of the liver, lung and kidney tissues of twenty-six normal guinea-pigs was studied. There was no pronounced difference between the sexes. A further series of twenty-four animals that were kept in the dark, one-half receiving ultraviolet radiation from a mercury vapor lamp, were similarly studied. No progressive change was apparent in either group as compared with the normal. A series of forty guinea-pigs were inoculated with suspensions from the same strain of tubercle bacilli. Twenty-eight were infected at the first attempt, and nine showed no sign of disease post mortem. The infected animals were killed at different stages of the disease. There was a definite sustained decrease in the lipolytic activity of the kidney tissues as the disease spread. In the liver there was an early pronounced drop in lipolytic activity, a partial recovery during the period of early tubercle formation in the organ, and a final sinking away. The extent of this late decrease was probably more apparent than real. The lung tissues, however, showed a definite progressive increase in lipolytic activity, which still revealed an upward tendency during the period of early pulmonary involvement.

AUTHORS' SUMMARY.

THE SIGNIFICANCE OF TISSUE CULTURE FOR PATHOLOGIC PHYSIOLOGY. A. FISCHER, Verhandl. d. deutsch. path. Gesellsch. **26**:67, 1931.

Tissue culture permits one to study the functions of the living cells in vitro. By the isolation and cultivation of cells that constitute diseased tissue the various disease processes may be better understood than by histologic methods.

In the study of infectious diseases, tissue culture is extremely valuable for analyzing the reaction of cells and body fluids against bacteria. More important still is the possibility of cultivating ultravisible virus together with living cells. Many viruses are not living organisms, but substances produced by tissue cells or blood cells; it is apparently impossible to cultivate a virus in dead material. Carrel cultures of chick embryo tissue, when inoculated with from 25 to 250 units of the virus of variola, will yield from 10,000 to 100,000 units in one week. The virus of Rous' chicken sarcoma can be cultivated for an unlimited period of time with embryonic chicken tissue. The cultivation of virus in pure cultures of tissue cells will open a new field for the study of the nature of viruses, whether they are micro-organisms or organic substances.

After the microscopic, bacteriologic and experimental era of the study of cancer, tissue culture is at present the most promising method of approaching the unsolved problems. The existence of a specific cancer cell is proved by the tissue culture, since cells isolated from different carcinomas and sarcomas and cultivated in pure cultures preserve their malignant properties and produce malignant tumors after transplantation into animals. In carcinoma as well as in sarcoma the malignancy is dependent on one cell type and there is no proof that other cells (macrophages) are necessary for the activation of the malignant principle.

Several characteristics of cancer cells in vitro help to explain the unlimited proliferation of malignant cells in the body. Cancer cells proliferate well in pure plasma or serum, while normal cells cease to grow under these conditions. The growth of malignant cells is activated when normal tissue cells are added to the culture. The normal tissue is invaded in vitro by the cancer cells and finally is destroyed. Under optimal conditions, cancer tissue grows more slowly than normal tissue, but the incidence of mitoses in cultures of carcinoma is ten times higher than in the culture of normal cells. This is explained by the fact that the average duration of life of cancer cells is extremely short. The death of cancer cells is compensated by increased proliferation of adjacent cells. The mechanism of tumor growth seems to be the same as that of regeneration. By simple mechanical lesions, a culture of normal cells can be stimulated to abundant growth, even if embryonic extract is omitted. The greater vulnerability of cancer

cells can be easily demonstrated in vitro; the cause of it is, however, unknown. The unlimited proliferation of tumor cells in the body is regarded by Fischer as a physiologic sequel of the vulnerability and the short life of cancer cells, and it resembles the regenerative processes following mechanical injuries of normal cells in tissue cultures.

C. ALEXANDER HELLWIG.

INFLUENCE OF CHEMICAL SUBSTANCES ON CELLS IN TISSUE CULTURE. F. DEMUTH, Verhandl. d. deutsch. path. Gesellsch. **26**:95, 1931.

Different chemical substances were added to tissue cultures in isotonic solutions, and their influence on the size of the cells and the nuclei and on the intensity of the growth was studied. More than 7,000 cells were measured. Calcium chloride does not change the size of the culture area, but causes a marked decrease in the size of the cells and a lesser decrease in that of the nucleus. Sodium bicarbonate, when added to calcium chloride, inhibits the calcium effect, while carbon dioxide increases it. Hydrogen ions decrease the size of the cells and that of the nuclei, while hydroxyl ions enlarge the size of the cells and enlarge less that of the nuclei. The hydroxyl ions inhibit the growth of normal, but not that of carcinomatous, tissues. Manosodium acid phosphate reduces the size of the nuclei and that of the cells. Disodium acid phosphate enlarges the normal cells, but decreases the size of carcinoma cells. Potassium enlarges only the cancer cells and inhibits the growth of normal and malignant epithelial cells. Sodium bicarbonate interferes with the growth of carcinoma tissue and enlarges carcinoma cells. Magnesium causes changes only in the cell structure. Lactic acid is without any noticeable effect. Dextrose in higher concentration is detrimental to normal tissue, but stimulates the growth of carcinoma tissue and enlarges cancer cells. Collargol decreases the size of fibroblasts, but not that of epithelial cells. Copper sulphate reduces the sizes of all kinds of cells, but inhibits the growth only of carcinoma tissue under certain conditions. Medium concentrations of heavy metals are often more effective than higher ones.

C. ALEXANDER HELLWIG.

EXPERIMENTAL ERGOSTEROL POISONING. M. HAENDEL and J. MALET, Virchows Arch. f. path. Anat. **276**:1, 1930.

The administration of irradiated ergosterol to rabbits and guinea-pigs caused cachexia that was associated with cholesteremia and with lipoid infiltration of the organs. The tissue changes were more marked in castrated than in normal animals. The toxic action of irradiated ergosterol is the same as that of cholesterol.

W. SAPHIR.

Pathologic Anatomy

THE DEGENERATION OF ARTICULAR CARTILAGE IN BOVINE JOINTS. G. A. BENNETT and W. BAUER, Am. J. Path. **7**:399, 1931.

Constant differences in the synovial fluid of the carpometacarpal and astragalotibial articulations of the cow have been described in a previous publication. The finding of areas of degeneration in the articular cartilages of the carpometacarpal articulations of all cattle over 2 years of age would appear to be an adequate explanation of these differences. These areas of progressive degeneration in articular cartilage have been studied systematically, and the successive changes have been described and illustrated. The development of the carpometacarpal articulations was studied in a series of bovine embryos and calves. The vascular articular cartilages of embryos and calves became avascular before the animals attained the age of 2 years. Pronounced rearrangement of the subchondral bone trabeculae resulted in a relatively deficient bony support of the medial articular cartilage where the degenerative lesions occur. The possible etiologic factors of

such lesions of cartilage have been discussed. It was concluded that they were probably due to repeated trauma in weakly constructed articulations. Deficient subchondral bone support was thought to be an important predisposing factor. The type of lesion of cartilage described in this paper is not wholly similar to any of the joint lesions described in human arthritis.

AUTHORS' SUMMARY.

THE INDUCED DEVELOPMENT AND HISTOGENESIS OF PLASMA CELLS. F. R. MILLER, J. Exper. Med. **54**:333, 1931.

As the result of finding numerous plasma cells in the omenta of rabbits that had received injections of tuberculoprotein, a method to induce the production of large numbers of these cells has been discovered. The tissues in which they were pronouncedly increased were the subserosal connective tissues of the omentum, body wall and intestinal wall. The precursor of the plasma cells is a primitive connective tissue cell. As this cell develops into the typical Marshalkó plasma cell there is a progressive increase in the basophilia of the cytoplasm, the nucleus becomes eccentric, a condensation of the chromatin occurs near the nuclear membrane, and there is a loss of the nucleoli. At the time when the nucleus assumes the eccentric position, the clear area appears in the center of the cytoplasm. The early cells are capable of reproducing themselves by mitosis, while the typical mature cells divide by amitosis. The mature plasma cells often have muddy, spongy cytoplasm, which contains acidophilic or hyaline granules, as the cells grow old or begin to degenerate. The cells with granules or hyaline bodies usually have pyknotic or fragmented nuclei. These cells are the final stage reached by some plasma cells. Others, when degenerating, show vacuoles and signs of senility. Those with the granules and hyaline bodies are the so-called Russell body cells. Plasma cells developed in greatest numbers after the largest injections of tuberculoprotein. The differentiation into young, mature and senile forms was most clearly recognizable when some days had been allowed to elapse after the last large injection of the stimulating agent. A description of the plasma cell as viewed supravitally has been given. The cells are met with in the blood stream as well as in the tissues. They are characterized by their deep yellowish-gray cytoplasm, indistinct, eccentrically placed nuclei and large numbers of mitochondria. The plasma cells differ from lymphocytes, in that they do not develop in large numbers after direct stimulation of the lymph nodes with tuberculoprotein. The young plasma cells also differ in morphology from the young lymphocytes. When plasma cells were found in the lymph nodes, they were in the connective tissue cords. The plasma cell is a definite entity, having a maturation cycle. It is stimulated to great proliferation by certain toxic irritants.

AUTHOR'S SUMMARY.

THE HISTOPATHOLOGY OF NUTRITIONAL ENCEPHALOMALACIA OF CHICKS. A. WOLF and A. M. PAPPENHEIMER, J. Exper. Med. **54**:399, 1931.

Whatever may be the nature of the nutritive error or deficiency that in chicks is responsible for encephalomalacia, the immediate cause must be looked for in some agent or condition that impairs the capillary circulation of the brain. The essential lesion is an ischemic necrosis, followed, if the animal survives, by reparative organization of the dead tissue.

AUTHORS' SUMMARY.

THE MORPHOLOGY OF THE SO-CALLED ARGENTAFFINE CELLS IN THE PANCREAS. J. M. LASOWSKY, Frankfurt. Ztschr. f. Path. **41**:1, 1931.

This article deals with the demonstration of argentaffine cells in the pancreas of the dog under various conditions. The argentaffine cells show no affinity to chromic salts. The pancreas of the normal animal always contains argentaffine cells, which under normal circumstances do not vary in number. Secretin and pilocarpine, however, injected into dogs intravenously and intramuscularly respectively, led to an increase of the argentaffine cells. Because of the similarity of

the secreting cells within the pancreas and the argentaffine cells, it is possible that they are related genetically. In atrophic conditions of the pancreas, the number of the argentaffine cells is markedly increased, owing to a transformation of the atrophic parenchymal cells into argentaffine cells. Because of the facts that the atrophic gland showed no islands of Langerhans and that there was no change in the carbohydrate metabolism, the author assumes that the dying cells of the islands are compensated for by argentaffine cells.

OTTO SAPHIR.

LIPOID CELL HYPERPLASIA OF THE SPLEEN. H. WILLE-BAUMKAUFF, Frankfurt. Ztschr. f. Path. **41**:14, 1931.

Lipoid cell hyperplasia of the spleen is found in cases of marked jaundice due to compression or occlusion of the common duct, as well as in cases of diabetic lipemia and in arteriosclerosis. The principal cause of the changes in the spleen is an increase of lipoids in the circulating blood. The storage of lipoid substance occurs to the greatest extent within the spleen. Some authors believe that there is also a moderate involvement of the reticulum cells in the bone marrow and the mesenteric lymph nodes and of the Kupffer cells in the liver. Cases in which an enlargement of the spleen and marked morphologic changes in the organ are shown must be differentiated from those in which this organ contains only a moderate number of fat-containing or lipoid-containing cells. One should distinguish, therefore, between (1) cases in which there are only a few lipoid cells in the spleen (probably a frequent finding); (2) those in which there is lipoid cell hyperplasia (W. H. Schultze) and sometimes a large splenic tumor, and (3) cases of Niemann-Pick's disease and Gaucher's disease.

AUTHOR'S SUMMARY.

THE PATHOLOGIC ANATOMY OF GAUCHER'S DISEASE. A. ANTONOW, Frankfurt. Ztschr. f. Path. **41**:26, 1931.

Eight cases of Gaucher's disease are reported. The opinion is expressed that the Gaucher cells arise from the reticulum cells in the spleen, lymph node and bone marrow and from the adventitial elements of the fine blood vessels within the liver and lymph nodes. The Gaucher cells in the liver are found at first in the region surrounding the central vein and later in the periphery of the hepatic lobule. It is not impossible that Gaucher cells in the liver may also be derived from the hepatic cells. Young forms of Gaucher cells in the bone marrow have an iron-containing pigment, which later disappears. Gaucher's disease is an expression of a metabolic disorder combined with a storage of a special cerebroside-cerasin within the adventitial cells of the spleen, liver, lymph nodes and bone marrow. The author believes that the occurrence of Gaucher cells not only in these organs, but also in other organs, such as the tonsils, thymus, lung, suprarenals, etc., is proved.

OTTO SAPHIR.

THE PATHOLOGIC ANATOMY OF MULTILOCULAR ECHINOCOCCUS CYST IN THE LIVER. A. POSSELT, Frankfurt. Ztschr. f. Path. **41**:45, 1931.

In a large series of cases, the gross morbid anatomy of the multilocular echinococcus cyst in the liver is described. The author believes that the term "multilocularis" gives an erroneous conception, and prefers the term "alveolaris." The paper goes into many detailed descriptions which cannot be covered by abstract.

OTTO SAPHIR.

THE CHANGES OF THE ARTERIES IN INCREASING AGE. A. M. TROITZKAJA-ANDREEWA, Frankfurt. Ztschr. f. Path. **41**:120, 1931.

The arteries of the elastic type reveal very early many coarse collagenous fibers. These are found in the media, are circular in distribution, and show anastomoses.

With increasing age, they become thicker and also more numerous. The arteries of the muscular type show, at first, only a few fine collagenous fibers between the muscle elements; later, however, the fibers become thicker and increase in number. The arteries of the elastic type reveal a decrease in the muscle elements in old age. Through the increase of the collagenous fibers, the regular arrangement of the muscle fibers is disturbed. The collagenous fibers in both types of arteries become indistinctly stained with increasing age. Their outlines are vague. This change is spoken of as "hyalinosis" (Voigt). There is no relation between arteriosclerotic intimal changes and the proliferation of collagenous fibers in the media. On account of the lack of clinical data, nothing can be said as to a possible relationship between the changes described in the arteries and changes in the blood pressure.

OTTO SAPHIR.

Pathologic Chemistry and Physics

CHEMISTRY OF THE BLOOD IN THROMBO-ANGIITIS OBLITERANS (BUERGER). MAE FRIEDLANDER and SAMUEL SILBERT, Arch. Int. Med. **48**:500, 1931.

A chemical analysis of the blood of forty patients with thrombo-angiitis obliterans was made to determine the total ash, total protein, calcium, phosphorus, chloride, sugar and cholesterol content. Increases in the total ash, total protein, calcium and cholesterol content were noted. No striking abnormalities were found in the values determined for chlorides and sugar. Tests for tolerance of sugar gave normal results. The conclusion that there is a tendency to concentration of the blood in thrombo-angiitis obliterans seems warranted.

AUTHORS' SUMMARY.

PROTEOLYTIC ACTIVITY OF THE SPLEEN. A. STRAUSS, Beitr. z. path. Anat. u. z. allg. Path. **85**:251, 1930.

The proteolytic activity of spleen pulp was determined by subjecting Loeffler blood serum plates to the action of the pulp placed on the surface of the coagulated serum with a standard loop. A relative quantitative estimate of the proteolytic activity was obtained by noting the time at which evidence of digestion could first be detected and the depth of the digested area. The reaction optima were found to be p_H 8 and p_H 5, proteolysis being somewhat more active at the more alkaline reaction. Splenic tissue was lytic also for coagulated egg white and gelatin. Proteolytic activity varies somewhat with the time after death at which the spleen is removed. It begins to increase about two hours after death and reaches a maximum from one to three days after death. The maximum activity is dependent on the initial enzyme content of the tissue at the time of death, rather than on the time after death. The object of the study was to find an explanation of the softening that occurs in the enlarged spleen of acute infections, and that is sometimes seen in the enlarged spleen following acute hemorrhagė. The proteolytic activity of the spleen lies at the upper limit of normal in the early stages of septic swelling and reaches its maximum in the fully developed acute splenic tumor. It is highest just before the softening of the swollen spleen occurs. The softening is therefore apparently due to autolysis of the pulp by proteolytic enzymes. As the splenic tissue again becomes more firm, the proteolytic activity of the tissue returns to normal limits. The swollen posthemorrhagic spleen is also more proteolytic than normal, but the increase is not as great as in the septic spleen. The tissue of the spleens of children from 3 to 5 years of age and of the indurated and atrophic adult spleen exhibited no increase in proteolysis, suggesting that the structure of the spleen is a factor in the process. Proteolytic activity is ascribed to the enzymes derived from leukocytes contained in the splenic pulp. These enzymes are activated by the blood serum and perhaps also by autolytic and bacterial products. That leukocytes are necessary is established by the fact that the spleens of animals and of children, which contain few or no leukocytes,

and the spleen in a case of agranulocytosis had little proteolytic activity. Absence of proteolysis in the spleen in myeloid leukemia is cited as evidence for the necessity of activation of the leukocytic enzymes. Failure of the spleen to enlarge and soften in acute peritonitis is ascribed to a possible filtrative action of the liver.

O. T. SCHULTZ.

THE OCCURRENCE OF INSULIN IN URINE. K. UEBERRACK and T. ZELL, Biochem. Ztschr. **239**:42, 1931.

Insulin added to urine can be demonstrated in experiments. The urine of insulin-resistant patients and of diabetic patients given too much insulin does not contain insulin.

WILHELM C. HUEPER.

COPPER IN HUMAN AND BOVINE MILK. S. G. ZONDEK and M. BANDMANN, Klin. Wchnschr. **10**:1528, 1931.

The copper content of woman's milk is on the average three times as great as that of cow's milk (from 0.5 to 0.6 mg. as contrasted with from 0.15 to 0.2 mg. per liter). Formula mixtures of cow's milk for infants are also low in copper content and differ little in this respect from the undiluted cow's milk. The difference in copper content in cow's and woman's milk is about 0.4 mg. per liter. In formula feeding, copper should be added.

AUTHORS' SUMMARY.

DETERMINATION OF NONPROTEIN NITROGEN BY DIRECT NESSLERIZATION. A. LUBLIN, Ztschr. f. phys. Chem. **200**:1, 1931.

The turbidity sometimes appearing after the addition of Nessler's reagent to the ashed material (Folin's method) is due to a reaction of the reagent with copper sulphate and the sodium tungstate used for the deproteinization. As this turbidity interferes with the colorimetric reading, the following modification in procedure is recommended: The copper sulphate may be replaced by a few drops of hydrogen dioxide and the Folin-Wu reagent by 20 per cent trichloracetic acid, which latter can also be used for oxalated blood (from 1.5 to 5 cc.). For the ammonium sulphate solution used as standard, a urea standard solution is substituted. The urea solution, however, must also be ashed. About ten minutes are required for the determination of nonprotein nitrogen with this method.

WILLIAM C. HUEPER.

OXALIC ACID IN BLOOD. W. MEY and S. MANGERI, Ztschr. f. phys. Chem. **200**: 31, 1931.

A method for the determination of oxalic acid in blood is described. The error range of the method is 4 per cent. Human blood contains 3 mg. per hundred cubic centimeters oxalic acid; rabbits and horse blood, from 6 to 9 mg. per hundred cubic centimeters.

WILHELM C. HUEPER.

NONPROTEIN SULPHUR IN BLOOD, PLASMA AND SERUM AND GLUTATHIONE SULPHUR IN BLOOD. J. ST. LORANT, N. HAJDIS and W. WEIL, Ztschr. f. phys. Chem. **200**:121, 1931.

Tunnicliffe's method for the determination of glutathione is unreliable. Therefore it was attempted to estimate glutathione indirectly through determinations of sulphur in total blood, plasma and serum. The sulphur content of plasma or of serum is practically constant in healthy persons (2.5 mg. per hundred cubic centimeters). Caro's reaction was used. In total blood, the sulphur content ranges between 6.11 and 7.30 mg. per hundred cubic centimeters. Glutathione sulphur is calculated from these figures to be between 5.42 and 4.30 mg. per hundred cubic centimeters in men and between 5.30 and 4.30 mg. per hundred cubic centimeters in women or an average of 4.86 and 4.80 mg. per hundred cubic centimeters, respectively.

WILHELM C. HUEPER.

Microbiology and Parasitology

TUBERCLE BACILLI IN CHILDREN WITH ERYTHEMA NODOSUM. ARVID WALLGREN, Am. J. Dis. Child. **41**:816, 1931.

Children with erythema nodosum who give positive tuberculin reactions often excrete tubercle bacilli. This proves that the enlarged hilar shadows in the children giving the positive reactions are of tuberculous etiology, and that the children really are suffering from tuberculosis; this ought to be regarded as an argument in favor of the tuberculous nature of the erythema. At the same time, these researches yield the valuable information that children with erythema nodosum are often spreaders of infection, and that other children ought to be protected from contamination by them. Children with erythema nodosum should not go to school for a certain length of time after the disease has developed, and if they are admitted to a hospital they should be isolated.

AUTHOR'S SUMMARY.

MENINGITIS DUE TO BACILLUS ACIDI-LACTICI IN A NEW-BORN INFANT. HARRY D. PASACHOFF, Am. J. Dis. Child. **41**:862, 1931.

A case of meningitis caused by Bacillus acidi-lactici in a new-born infant is reported. A review of the literature shows that only three cases due to this organism have previously been reported, two in new-born infants and one in an adult. Some of the possible modes of entry of the infection are considered.

AUTHOR'S SUMMARY.

THE BLOOD PICTURE IN WHOOPING COUGH. L. W. SAUER and L. HAMBRECHT, Am. J. Dis. Child. **41**:1327, 1931.

After initial and terminal leukopenia had been noted in the pertussis cycle, the protocols of our experimental pertussis in young monkeys were examined. Five of the seven animals in which paroxysmal coughs developed showed an initial leukopenia, and three of the four that were not killed for histologic study showed a terminal leukopenia. This evidence is further proof that the coughs of the animals were due to infection with Bacillus pertussis. The blood picture is seldom an aid in early diagnosis. Initial leukopenia and terminal leukopenia are probably integral parts of the blood picture in pertussis. Leukocytosis and lymphocytosis are usually present when the paroxysmal stage is well established.

AUTHORS' SUMMARY.

A CHEMICAL VIEW OF THE PATHOGENESIS OF TUBERCULOSIS. ESMOND R. LONG, Am. Rev. Tuberc. **22**:467, 1930.

The first element in the pathogenesis of tuberculosis is the capacity of the bacillus to multiply in the physical and chemical environments of the tissues of the host. The growth requirements of the tubercle bacillus in artificial culture are well known. Its water relations, respiration and dependence on glycerol are distinctive. In general, animal tissues are well adapted to meet these requirements of growth. The reactions and the carbon dioxide production of the tissues may reach an inhibiting degree. The availability of glycerol may be a factor. Modifying the glycerol content of tissues artificially has a profound effect on growth in experimental tuberculosis. Growth in the animal body is usually intracellular. Its rapidity depends on the animal. Intense initial cellular response does not tend to check subsequent progression of the disease. Native immunity in man or animals appears to depend on the capacity of tubercle bacilli to multiply in the cells of the host infected. Acquired immunity probably depends on a similar factor, and is usually associated with hypersensitiveness. The latter feature profoundly affects the anatomic character. The manifestations of hypersensitiveness are a

response to the proteins of the tubercle bacillus. The necrosis of the tubercle and the acute cellular responses of tuberculosis are largely protein effects. The lipoids are responsible for much of the cellular response in its initial and chronic manifestations.

H. J. CORPER.

ANATOMICAL CHARACTERISTICS OF TUBERCULOSIS IN JAMAICA. EUGENE L. OPIE, Am. Rev. Tuberc. **22**:613, 1930.

A small group of autopsies made on persons of the Negro race in Jamaica are described because they furnish anatomic evidence of the acute character of the pulmonary tuberculosis prevalent in Jamaica. Observations made on dispensary patients showed that the average duration of the disease in Jamaica, as elsewhere, varies with the age at death.

H. J. CORPER.

THE EFFECT ON GROWTH OF THE TUBERCLE BACILLUS OF MODIFICATION OF THE AMOUNT OF GLYCEROL IN THE TISSUES. ESMOND R. LONG and ARTHUR J. VORWALD, Am. Rev. Tuberc. **22**:636, 1930.

An attempt was made to apply the knowledge of the nutrition of the tubercle bacillus in laboratory culture to the growth of the micro-organism and the spread of tuberculosis in animals. Glycerol, fed or injected over long periods of time into tuberculous rats, appeared definitely to enhance the growth of tubercle bacilli in lesions, and to increase the extent of the disease, reproducing to some extent in vivo the conditions of growth in vitro. In the attempt to produce the opposite effect, a reduction of the amount of free glycerol available for bacillary growth in animal tissues, palmitic acid was administered in the largest amounts tolerated by the animal of the experiment, the theory being that an excess of fatty acid in the body would force a synthesis of some of the free glycerol present with the fatty acid administered, to form neutral fat, which does not support the growth of the tubercle bacillus. The results were not consistent, but in the majority of cases the extent of tuberculosis was less in the animals treated with palmitic acid than in the controls. The fatty acid was deleterious to the general health of the animals treated. An unexplained finding was an increase in the number of visible acid-fast bacilli in the animals treated with palmitic acid, in spite of a decrease in the extent of the tuberculosis.

H. J. CORPER.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN. FLORENCE B. SEIBERT and BETTY MUNDAY, Am. Rev. Tuberc. **23**:23, 1931.

The tuberculin action of tuberculin protein, as seen, for example, in the skin reaction in tuberculous animals, appears to be due to a specific portion of the protein molecule, which seems to be present in the first, but not the subsequent, cleavage products obtained during hydrolysis of the protein. The smallest protein molecule in tuberculin capable of giving the reaction has probably a comparatively low molecular weight, of from 1,000 to 2,400 or less, as indicated by passage through membranes. Only the protein and possibly its first cleavage products produce the precipitin reaction in serums from animals immunized with the protein-carbohydrate complex (that is, the whole ammonium sulphate precipitate of tuberculin prepared from the human type of tubercle bacillus). The lethal power of the tuberculin in tuberculous animals and in normal animals is probably associated with the very early cleavage products of the protein. Polysaccharide is chemically associated chiefly with the early cleavage products of the tuberculin protein and cannot be removed by washing, but is released at p_H 4.8. Nearly one fourth of the polysaccharide in tuberculin is so associated. The rest of the polysaccharide, not bound to the protein, passes even the finest filter used in these experiments. With this information it has been possible to prepare in quantity an extremely potent tuberculin protein in the form of a powder, which is practically completely water-soluble

and therefore undenatured, and which has a nitrogen content of 16 per cent and a polysaccharide content of 2.7 per cent or less. Only 0.01 mg. is required to produce a maximum skin reaction in sensitive tuberculous guinea-pigs, and 5 mg. is sufficient to kill such a guinea-pig in twenty-four hours. It gives a very high precipitin titer with serums from rabbits immunized with the protein-carbohydrate complex of tuberculin (that is, the whole ammonium sulphate precipitate of tuberculin prepared from the human type of tubercle bacillus). The primary toxicity for normal guinea-pigs is extremely low — about that of timothy bacillus protein.

H. J. CORPER.

EXPERIMENTAL TUBERCULIN PNEUMONIA. ALFRED LARSON and ESMOND R. LONG, Am. Rev. Tuberc. **23**:41, 1931.

The authors used a purified tuberculin prepared by ultrafiltration from a human culture on Long's synthetic medium and tested this by intratracheal injection into tuberculous and nontuberculous guinea-pigs. The sharp differentiation expected in the effect of tuberculin protein on the lungs of normal and tuberculous animals was not realized. In the alveoli of both groups a cellular exudate was produced. A quantitative difference in the normal and sensitized animals was found, however: the exudate caused by the tuberculin tended to clear up in from forty-eight to seventy-two hours, leaving the lung normal, and in no instance was caseous necrosis noted.

H. J. CORPER.

MONOCYTES IN TUBERCULOUS ANIMALS. J. T. GEIGER, Am. Rev. Tuberc. **23**:76, 1931.

In the normal nontuberculous animal there is no reservoir of monocytes capable of supplying great numbers of these cells on acute demand. Such a reservoir can be and is established in tuberculous animals. After its establishment the monocytes respond to acute stimuli in a manner similar to that of the granulocytic leukocytes.

H. J. CORPER.

DO BROTH CULTURE FILTRATES CONTAIN A BACTERIAL GROWTH-INHIBITING SUBSTANCE? L. A. BARNES, J. Bact. **21**:395, 1931.

The results of the experiments described do not support the observations of Besredka that filtrates of broth cultures contain a substance, liberated from the microbial cell, that inhibits bacterial growth. When filtrates prepared from broth cultures, pancreatin-digested broth or bacterial cell solutions are added in varying proportions to nutrient broth, the resulting diminution in the growth of the inoculated organisms is comparable to, or even less than, that observed when distilled water (or saline solution) is used as the diluent. It appears to the writer, therefore, that the effect of culture filtrates on the growth of bacteria is due to an alteration of necessary nutrient materials rather than to the presence of an inhibitory agent.

AUTHOR'S SUMMARY.

THE ETIOLOGY OF INFECTIOUS DIARRHEA (WINTER SCOURS) IN CATTLE. F. S. JONES and R. B. LITTLE, J. Exper. Med. **53**:835, 1931.

A disease of cows manifested by severe diarrhea is described. The condition is characterized by the frequent passage of dark brown or black feces, often containing mucus and blood. The principal lesions are catarrhal inflammation of the small intestine and degeneration of the liver. By feeding feces from cows spontaneously infected to calves a similar, but milder, disease, characterized by the same type of enteritis, was produced. Vibrios were cultivated from the inflamed intestinal tract in such experimentally induced cases. Pure cultures of the vibrios when fed to other calves, in certain instances, produced diarrhea and a well marked

enteritis similar to that observed both in the spontaneous disease and in calves following the feeding of feces from naturally infected cows. Vibrios were recovered from the inflamed small intestine of three of four animals fed such cultures.

AUTHORS' SUMMARY.

VIBRIONIC ENTERITIS IN CALVES. F. S. JONES and R. B. LITTLE, J. Exper. Med. **53**:845, 1931.

An intestinal disorder of calves is described. The clinical manifestations are usually observed in calves 2 or more weeks old. Our experiments indicate that infection may take place relatively early in life and may for a time produce only a mild reaction, but as the disease becomes more chronic, the clinical manifestations become more pronounced. The anterior portion of the jejunum is the primary locus of infection, but in more chronic cases practically the whole small intestine may be involved. Vibrios were cultivated from the inflamed intestinal mucosa in both the acute and the more chronic spontaneous cases. Vibrios were also obtained from the acutely involved intestines of young calves experimentally exposed to natural infection. On three occasions similar vibrios were found in cultures from the liver. When a single strain of vibrios that had been under cultivation for three months was fed to a young calf, subclinical infection was produced, and the organism was recovered. This strain after three passages on culture medium, when fed to a calf, produced a severe inflammation of the jejunum and ileum, and from these areas the organism was recovered.

AUTHORS' SUMMARY.

VIBRIOS (VIBRIO JEJUNI, N.SP.) ASSOCIATED WITH INTESTINAL DISORDERS OF COWS AND CALVES. F. S. JONES, M. ORCUTT and R. B. LITTLE, J. Exper. Med. **53**:853, 1931.

A number of vibrios obtained from the small intestines of calves fed feces from cows with spontaneous diarrhea, the natural intestinal disorders of calves, the experimentally induced infections of calves and cultures obtained from Dr. Theobald Smith were studied. From the close morphologic resemblance of the cultures, the similarities in motility, position and number of flagella, the tinctorial properties, and the tendency to fragmentation in older cultures, as well as the narrow nutritive requirements, we are led to regard them as a closely allied group, and we propose the name Vibrio jejuni. Immunologically, as judged by agglutination, the organisms have been divided into two groups, the smaller representing two strains originating from diarrhea in cows and the larger comprising one from this source and many from the disease in calves. The larger group can be subdivided by means of agglutinin absorption into cultures that do not contain the complete antigenic complex and others that do. Certain freshly isolated vibrios, when injected into rabbits, incite definite reactions terminating in a localization of the vibrios in the small intestine accompanied by catarrhal inflammation.

AUTHORS' SUMMARY.

BARTONELLA MURIS ANEMIA. D. PERLA and J. M. GOTTESMAN, J. Exper. Med. **53**:869 and 877, 1931.

The pathologic changes that follow a severe infection of the adult or young albino rat with Bartonella muris are of three kinds. First are those that result from the release of large quantities of cellular débris into the circulating blood. These include phagocytic activity and hyperplasia of the endothelial elements of the liver, thymus, lymph nodes and, in the young rat, of these elements in the spleen, with resultant capillary thromboses and focal necroses. Second, changes result from the anemia as such — fatty metamorphosis of the heart, liver and kidneys. Third, there is a severe nephrosis within some instances, a degenerative process in the glomeruli. In the bone marrow, hyperplasia of erythropoietic elements occurs.

In carriers of Bartonella muris certain changes are observed in the lymphoblastic, reticular and endothelial elements after splenectomy and recovery from the anemia. These changes appear from three to five months after splenectomy and are associated with immunity to further infection with Bartonella muris. The changes consist primarily of hyperplasia of hemolymph tissue, hyperplasia of the reticular and endothelial elements of lymph nodes, the formation of lymphoblastic foci periportally in the liver and peribronchially and perivascularly in the lung, regeneration of all elements of the thymus and marked hyperplasia of all elements of the bone marrow (increased hematopoiesis).

AUTHORS' SUMMARY.

THE ACCUMULATION OF IRON IN TUBERCULOUS AREAS. V. MENKIN and M. F. MENKIN, J. Exper. Med. **53**:919, 1931.

Daily intravenous injections of ferric chloride solution are followed by an accumulation of iron in tuberculous areas of the lungs. The iron accumulates in the caseous portions of the tubercles and is demonstrable by the prussian blue reaction. Quantitative determinations corroborate these results, and show that the iron content of lung tissue in tuberculous animals given injections of ferric chloride exceeds that in normal animals given injections of this salt, as well as that in tuberculous animals not given the injections.

AUTHORS' SUMMARY.

TICKS AS VECTORS OF TYPHUS FEVER. H. ZINSSER and M. R. CASTANEDA, J. Exper. Med. **54**:11, 1931.

Mexican typhus virus can be passed through ticks by the method of rectal injection. The virus remains alive in the ticks for at least twelve days. These studies, with one of our preceding publications and the work of Dyer, demonstrate that there are at least three insects — bedbugs, fleas and ticks — which must be considered as possibilities in the conveying of typhus fever from an animal reservoir to man. Our work will be continued by a study of rats and mice caught in typhus regions, such as Mexico City and its immediate vicinity, with a search for the virus in these rodents, as well as an analysis of the insects found on them or in the localities in which they are concentrated.

AUTHORS' SUMMARY.

TRANSMISSION AND CULTIVATION EXPERIMENTS WITH TRACHOMA. P. K. OLITSKY, R. E. KNUTTI and J. R. TYLER, J. Exper. Med. **54**:31, 1931.

Conjunctival tissue derived from alien and native American white persons having trachoma in an advanced stage has been used successfully to induce in monkeys of the species Macacus rhesus characteristic granular conjunctivitis. The transfer of infection was effected either by a single subconjunctival injection or by repeated swabbings with conjunctival secretions. Pathogenic strains of Bacterium granulosis have been recovered from the trachomatous tissues of six of eleven patients. In addition, the organisms have been isolated from the monkeys infected with the human material. Repeated swabbings with secretions obtained from monkeys having experimental trachoma have given rise to characteristic granular conjunctivitis in normal animals. In addition, repeated instillations of suspensions of fragments of conjunctival tissue derived from affected monkeys have led to a characteristic infection of the conjunctivae of normal monkeys. Contact infection occurs in monkeys, as it has long been known to occur in human beings, animals with smooth conjunctivae acquiring the experimental disease when merely caged with infected monkeys. Repeated instillations of cultures followed by rubbing of the eyelids lead to the disease in monkeys, a method of transfer that indicates one manner in which the disease may be transmitted from man to man. Yet another manner of producing the experimental condition is by repeated swabbings with cultures of B. granulosis. Noguchi has reported the successful outcome of the subconjunctival inoculation of cultures and the spread of the disease from an infected

conjunctiva to the other eye of the same animal. Tissues derived from human beings with trachoma or from monkeys having the experimental disease induce, on conjunctival inoculation into monkeys of the species Macacus rhesus, the same clinical and pathologic effects as do cultures of B. granulosis. The conjunctival lesions closely resemble, in clinical appearance and in microscopic changes, those of the follicular stages of trachoma in man.

AUTHORS' SUMMARY.

EXPERIMENTAL DERMAL PNEUMOCOCCUS INFECTION IN THE RABBIT. C. P. RHOADS and K. GOODNER, J. Exper. Med. **54**:41, 1931.

Experimental dermal pneumococcic infection in the rabbit is described in detail, and the histologic alterations are compared with those seen in the pneumonic lung in man. There would appear to be a basic similarity of the lesions in both tissues. A copious production of edema fluid is the outstanding characteristic of the early lesion. It occurs prior to any significant cellular change. In the spreading lesion an infiltration of the tissues with fluid precedes any other sign of reaction between tissue and microorganisms. It seems likely that the advancing fluid carries with it the infecting organisms and inoculates all tissues that it reaches. The resulting infection seems not to take place by an active invasion of micro-organisms, but by a progressive inoculation from an infected fluid.

AUTHORS' SUMMARY.

THE ACTION OF A BACTERIAL ENZYME ON TYPE III PNEUMOCOCCUS. O. T. AVERY and R. DUBOS, J. Exper. Med. **54**:51 and 73, 1931.

An organism has been isolated from peat soil which decomposes the specific capsular polysaccharide of pneumococcus type III. The isolation has been made possible by the use of a synthetic mineral medium containing the specific polysaccharide as the sole source of carbon. By repeated transfers in this medium the capacity of the organism to decompose the specific substance has been progressively increased. The organism is a pleomorphic bacillus, motile and spore-bearing, exhibiting metachromatic granules; its reaction to Gram's stain varies according to the medium on which it is grown. It is strictly aerobic, and grows well in plain broth and peptone solutions; it does not produce gas in any medium, and it forms small amounts of acid only on dextrine, galactose, lactose, salicin and trehalose; its growth is inhibited by dextrose. The organism decomposes the capsular polysaccharide of pneumococcus type III aerobically, between p_H 6.2 and 7.8, at room temperature and at 37.5 C., but not at 54 C. The decomposition of the specific substance is inhibited by the presence in the medium of other nutrients, such as peptones, which act as a more readily available source of energy. The action of the organism is specific; it does not attack the soluble specific substance of type I or type II, nor any of the other bacterial polysaccharides thus far tested. The organism possesses an endocellular enzyme. This enzyme has been extracted by autolysis of the bacterial cells; in sterile solution it exhibits the same specific action as do the organisms from which it is derived, decomposing only the capsular polysaccharide of pneumococcus type III. This enzyme decomposes the specific polysaccharide of type III under anaerobic, as well as under aerobic, conditions; it is inactivated at from 60 to 65 C.; the rate of decomposition of the specific substance is not affected by the presence of normal serum. There exists a quantitative relationship between the total amount of specific substance decomposed and the amount of enzyme preparation used; the existence of this relation makes it possible to express the activity of a given enzyme preparation in terms of the minimal amount required for the complete decomposition of a given amount of specific substance. The specific decomposition of the capsular polysaccharide of pneumococcus type III, by the organism as well as by the enzyme it produces, illustrates once more the specificity of the types of pneumococcus and confirms the fact that the capsular polysaccharides, and not some impurities carried along with them, are responsible for type specificity.

The bacterial enzyme that decomposes the purified capsular polysaccharide of pneumococcus of type III in vitro also destroys the capsules of the living organisms growing in culture mediums and in the animal body. Potent preparations of this same enzyme protect mice against infection with virulent type III pneumococcus. The protective action is type-specific. The protective activity of the specific enzyme is destroyed by heat (70 C. for ten minutes). The enzyme remains in an effective concentration for from twenty-four to forty-eight hours after its injection into normal mice. The enzyme has been found to exert a favorable influence on the outcome of an infection already established at the time of treatment. A definite relationship has been found to exist between the activity of the enzyme in vitro and its protective power in the animal body. The mechanism of the protective action is discussed with special reference to the relation between the decapsulation of the bacteria by the enzyme and the phagocytic response of the host.

AUTHORS' SUMMARIES.

PSITTACOSIS. T. M. RIVERS, G. P. BERRY and D. H. SPRUNT, J. Exper. Med. **54**:91, 105, 119 and 129, 1931.

Experimental Infection in Parrots (Rivers, Berry and Sprunt).—The virus of psittacosis is present in the nasal secretions, feces, blood, spleens and livers of infected parrots. Parrots are susceptible to intra-oral, intranasal or intramuscular inoculations of the virus. The most constant pathologic changes produced by psittacosis in parrots occur in the spleen and the liver. The lesions in the latter organ consist of areas of necrotic liver cells and damage to bile ducts. In no instance, in our experience, were lesions observed in a parrot's lungs comparable to those found in the lungs of man. "Minute bodies" similar to those described by Levinthal and others were found in many, but not in all, of the infected birds. Parrots that have recovered from one attack of psittacosis exhibit an active immunity against reinfection.

Experimental Infection in Mice (Rivers and Berry).—The work presented in this communication concerning psittacosis in mice confirms Krumwiede's observations that mice inoculated intraperitoneally with emulsified livers and spleens containing the virus acquire the disease, and that the malady can in this way be passed serially through a number of mice. Furthermore, it has been shown that mice are susceptible to the virus administered intracerebrally, and that the active agent can be propagated indefinitely by means of brain to brain inoculations. Moreover, by the use of mice, the presence of the virus of psittacosis in the sputum of a patient with the disease has for the first time been demonstrated. It follows that the mouse is available for diagnostic purposes. The pathologic findings in infected mice consist of enlarged fatty livers that frequently show areas of necrosis infiltrated with polymorphonuclear and mononuclear cells; enlarged spleens with areas of necrosis and cellular infiltrations involving the pulp and lymphoid follicles, and, finally, intracerebrally infected animals, a meningo-encephalitis. The "minute bodies" described by other observers were not found in all animals, but they were seen with sufficient frequency in smears of peritoneal and meningeal exudates and in smears and sections of livers and spleens to demand serious consideration as the possibile etiologic agent of the disease. Neutralizing and protective antibodies were not found in convalescent human serums when the mouse was used as the test animal.

Experimental Infection in Rabbits and Guinea-Pigs (Rivers and Berry).—Rabbits and guinea-pigs are susceptible to psittacosis virus introduced intracerebrally. By means of brain to brain passages in these animals the active agent is capable of propagation indefinitely. Serial passages of the virus through rabbits and guinea-pigs do not cause the active agent to lose its pathogenicity for parrots and mice. The chief clinical evidences of infection in rabbits and guinea-pigs following intracranial inoculation of the virus are fever and loss of weight. The pathologic changes are characterized by a mild meningo-encephalitis, and fatty

degeneration, focal necrosis and infarction of the liver. Rabbits on recovery from an attack of psittacosis are actively immune. Two strains of virus, human and parrot, were found to be immunologically similar. No evidence was obtained to show that human convalescent serum possesses an appreciable amount of neutralizing substances.

Experimental Infection in Monkeys (Rivers and Berry).—The virus of psittacosis inoculated intratracheally or intranasally in monkeys produces a pneumonia similar to that caused by the same active agent in man. Intracerebral inoculation of the virus induces a meningo-encephalitis characterized principally by a mononuclear reaction in the meninges. Indirect evidence has been adduced to show that the portal of entry of the virus in man is the upper respiratory tract.

AUTHORS' SUMMARIES.

SPORES OF CLOSTRIDIUM TETANI. T. J. MURRAY and M. R. HEADLEE, J. Infect. Dis. **48**:436, 1931.

Effective temperatures for the destruction of spores of Clostridium tetani in physiologic solution of sodium chloride varied from 105 to 95 C. The time varied from five to sixty minutes, three strains resisting heating for sixty minutes at 95 C. Effective temperatures for dry spores varied from 140 to 125 C., and the time from five to forty minutes. Dried spores heated under moist conditions showed no increase in resistance. Spores in buffer solutions showed the best resistance at p_H 7. Spores in 1 per cent peptone solution were also more resistant at p_H 7. Increasing amounts of organic matter (peptone) tended to increase the thermal death time at p_H 7. Spores suspended in 2 per cent sodium chloride showed the greatest resistance to heat.

FROM AUTHOR'S SUMMARY.

SPORES OF BACILLUS ANTHRACIS. T. J. MURRAY, J. Infect. Dis. **48**:457, 1931.

Effective temperatures for the destruction of spores of Bacillus anthracis in physiologic solution of sodium chloride varied from 105 to 90 C. The time varied from five to forty-five minutes. Higher temperatures were necessary when dried spores were heated in the absence of moisture. Spores heated in buffer solution showed the best resistance at p_H 8. In 1 per cent peptone solutions the resistance increased as the p_H increased. The resistance of spores suspended in sodium chloride solutions decreased with the increase in the concentration of the salt.

FROM AUTHOR'S SUMMARY.

SPORES OF CLOSTRIDIUM WELCHII. M. R. HEADLEE, J. Infect. Dis. **48**:468, 1931.

The resistance of spores of Clostridium welchii in physiologic solution of sodium chloride varied from thirty minutes at 90 C., to ten minutes at 95 C., and five minutes or less at 100 C. Spores were more tolerant of acid than of alkali. Optimum resistance of spores was found in 3 per cent sodium chloride solutions. Spores in peptone and gelatin showed resistance inversely proportional to the concentration of organic substance. Optimum resistance was shown in 1 per cent starch solution. Icebox temperature had little effect on the thermal resistance of the spores. Dry spores were found more resistant to heat than wet spores.

FROM AUTHOR'S SUMMARY.

INFECTION WITH BRUCELLA ABORTUS. A. THOMSEN, J. Infect. Dis. **48**:484, 1931.

Two hundred and seventy-two persons in contact with cattle were examined for agglutinins and complement-fixing antibodies for Brucella abortus. Individuals in contact with sick animals showed a large percentage of positive reactions, but a history of undulant fever was noted in only one case. Complement-fixation tests gave more positive results than the agglutination test. Among the persons handling

flesh of killed animals and milk, few positive reactions were found. A control group of sixty-one healthy patients and nonfebrile patients showed no positive reactions.

EDNA DELVES.

OTITIS MEDIA AND MASTOIDITIS DUE TO STREPTOCOCCUS EPIDEMICUS. I. PILOT, M. LAMPERT and D. J. DAVIS, J. Infect. Dis. **48**:498, 1931.

Six cases of otitis media and four of mastoiditis due to Streptococcus epidemicus are described. The complications due to this streptococcus may follow sporadic sore throat, scarlet fever or mild infections of the upper respiratory tract.

AUTHORS' SUMMARY.

TONSILLECTOMY IN CARRIERS OF STREPTOCOCCUS EPIDEMICUS. I. PILOT and D. J. DAVIS, J. Infect. Dis. **48**:501, 1931.

Tonsillectomy in carriers of Streptococcus epidemicus causes rapid disappearance of this organism from the throat. Persons presenting sporadic cases of septic sore throat, otitis media and mastoiditis often become carriers of S. epidemicus in tonsillar crypts. S. epidemicus may cause acute infection in tonsillectomized persons, but the organisms disappear during convalescence. S. epidemicus was found in the crypts of 13.8 per cent of extirpated tonsils. All of the patients gave a history of sore throat. Many whose tonsils revealed S. epidemicus had cervical adenitis, arthritis or arthritic pains. The removal of the tonsils in these patients resulted in marked clinical improvement.

AUTHORS' SUMMARY.

SPORADIC CELLULITIS AND ABSCESS DUE TO STREPTOCOCCUS EPIDEMICUS. I. PILOT and D. J. DAVIS, J. Infect. Dis. **48**:505, 1931.

Cellulitis and deep subcutaneous abscess may be due to Streptococcus epidemicus. Abscesses in the cervical lymph glands may follow sore throat due to this organism. Peritonsillar abscess, suppurative otitis media, mastoiditis, meningitis and empyema may also be caused by S. epidemicus. These infections appear to be sporadic and not associated with milk-borne epidemics of sore throat.

AUTHORS' SUMMARY.

DISSOCIATION OF HEMOLYTIC STREPTOCOCCI. R. TUNNICLIFF, J. Infect. Dis. **48**:511, 1931.

Many strains of hemolytic streptococci from erysipelas, scarlet fever and septic sore throat remain stable for years, both immunologically and culturally. A few strains dissociate, and the cocci from the dissociated colonies differ from those in the original culture in colony formation, immunity reaction, sometimes in production of color on chocolate agar, and in virulence. Many of the dissociated cultures may revert to the original type of streptococcus.

AUTHOR'S SUMMARY.

EPIDEMIOLOGY OF UNDULANT FEVER. C. F. JORDAN, J. Infect. Dis. **48**:526, 1931.

Agglutinins for Brucella melitensis in the group of the general population studied appear to be due to the use of raw dairy products. The group of veterinarians show a higher percentage of positive agglutinin reactions, owing to their direct contact with cattle. Infection with Br. melitensis is in direct proportion to exposure. Infection due to the ingestion of raw dairy products seems dependent on the amount consumed, the duration of exposure and the number of organisms ingested. The number of "no agglutination" reactions in persons following infection associated with disease and infection without disease indicate the probable rôle of immunity in the epidemiology of undulant fever.

EDNA DELVES.

FECAL FLORA OF ADULTS. A. G. SANBORN, J. Infect. Dis. **48**:541, 1931.

The fecal flora was characteristic of the individual. This was particularly true of the Bacillus coli-Lactobacillus acidophilus ratio. The majority had L. acidophilus in the feces. Sporalating anaerobes, a marked evidence of proteolytic activity, usually accompanied a high count of L. acidophilus. Presumably nonsporulating anaerobes were found in considerable numbers. The frequent presence of a small, gram-negative, anaerobic bacillus which seemed to affect the ratio of gram-negative to gram-positive bacilli is mentioned. High and low dilution plates, incubated under increased carbon dioxide tension, were used.

EDNA DELVES.

BRUCELLA ABORTUS INFECTION. K. T. SASANO, D. CALDWELL and E. M. MEDLAR, J. Infect. Dis. **48**:576, 1931.

Serums of 1,000 persons revealed positive complement-fixation for Brucella abortus in 96 and positive aggluination in 78. In only five cases was the diagnosis of undulant fever made. In all these cases, complement-fixation was positive, and the titers of agglutination ran from 1:135 to 1:200. In rabbits the complement-fixing substances and the agglutinins developed at about the same time, but the former persisted longer. Complement-fixation and agglutination remain for years after apparent recovery from the infection. A diagnosis of undulant fever based on positive complement-fixation and low titers of agglutination in the absence of positive blood cultures, is doubtful.

EDNA DELVES.

BACILLUS HEMOLYTICUS. L. R. VAWTER and E. RECORDS, J. Infect. Dis. **48**: 581, 1931.

Agglutinins for Bacillus hemolyticus were specific for the species. One of the sixteen strains studied did not belong to the same agglutination group. Cross-agglutination of B. hemolyticus serums did not occur with antigens of B. chauvaei, B. welchii, Vibrion septique (types I, II and III) and three strains of B. sordelli. Agglutinating serums of other pathogenic anaerobes did not agglutinate B. hemolyticus. Antigens of B. novyi were unstable, but B. novyi serum failed to protect animals inoculated with B. hemolyticus.

EDNA DELVES.

DIPTHERIA TOXIN. M. E. MAVER, J. Infect. Dis. **49**:1, 1931.

Chemical tests indicate that a protein is synthesized simultaneously with the production of toxin by diphtheria bacilli cultured in a synthetic medium. Experimental evidence that the toxic fraction is identical with or associated with a protein is as follows: In an electric field both toxin and protein migrate to the anode at p_H 4.05 and to the cathode at p_H 3.75. The protein and the toxin are simultaneously destroyed by trypsin and pepsin. Less action in destroying the toxin on the part of neutral trypsin, as compared with alkaline trypsin, might be taken as evidence that the toxic fraction is a whole protein. The production of crystalline protein toxin will be conclusive evidence.

FROM AUTHOR'S SUMMARY.

FECAL FLORA OF ADULTS. A. G. SANBORN, J. Infect. Dis. **49**:37, 1931.

The degree of transformation of the fecal flora in an acidophil direction by means of a specific carbohydrate diet is dependent on the original presence of appreciable numbers of L. acidophilus and the stability of the flora already present. A diet high in carbohydrate and one high in meat protein tend to simplify the aerobic fecal flora, the one in the acidophil, the other in the colonic direction. Non-lactose-fermenting gram-negative bacilli developed in persons with a strongly colonic flora resistant to transformation. Proteolytic activity may be reduced by decreasing the meat protein and by increasing the lactose in the diet, even when

there is only a slight increase in L. acidophilus in the feces. L. acidophilus in the feces is more easily increased in persons under 40 years of age. Persons irregular in their defecations often eliminate large quantities of L. acidophilus. Foreign strains ingested in L. acidophilus milk completely suppressed native strains. After milk therapy was stopped, the foreign strains soon disappeared, with a return of the native L. acidophilus. L. bulgaricus was not isolated from the feces of three persons, but in one case L. bulgaricus milk appeared to decrease the number of native L. acidolphilus. It is suggested that in adults L. acidophilus milk may be more valuable therapeutically than an attempted transformation of the fecal flora by appropriate diets.

EDNA DELVES.

BACTERIUM GRANULOSIS IN RELATION TO TRACHOMA: ITS PATHOGENICITY FOR VARIOUS MONKEYS AND APES. CHARLES WEISS, Tr. Am. Acad. Ophth., 1930, p. 233.

Working with three strains of Bacterium granulosis provided by the Rockefeller Institute, and later with two others isolated by ourselves, we have been able to produce only a transient type of follicular conjunctivitis, lasting not more than four months, in various types of monkeys and apes. We employed twenty-eight rhesus monkeys, two of the genus Macacus inuus (Algerian magots), one baboon, one callitriche and two chimpanzees. Of the twenty-eight rhesus monkeys, eight developed granular conjunctivitis. The two Algerian magots developed similar follicles. One baboon was inoculated three times, and transient follicles were induced twice, while the callitriche, which was inoculated once, remained free from infection.

In two young chimpanzees inoculated with the Rockefeller cultures of Bacterium granulosis only transient follicles were induced, whereas in a third young chimpanzee infected with fresh "virus" obtained from human trachomatous eyes advanced lesions developed, which contained Prowazek-Halberstaedter bodies, and both clinically and histologically showed a striking resemblance to the lesions of active human trachoma. Thus, eighteen days after infection, the mucous membranes of the lower conjunctiva were thrown into folds; there were papillary hyperplasia and hypertrophy, secretion and ptosis of the upper lid. The infection spread spontaneously to the uninoculated eye, and follicles developed in the upper and lower conjunctivae of both eyes, including the tarsi and retrotarsal folds. Attempts to isolate B. granulosis from the material removed from the conjunctival lesions of the patient, as well as from those of the chimpanzee, were unsuccessful. Histologic sections of the conjunctivae of the inoculated eye of this chimpanzee, taken on the two hundred sixteenth day after injection (when the animal died of generalized tuberculosis), showed roughening of the conjunctival surfaces, epithelial invaginations, lymphocytic infiltration, young fibroblasts and new connective tissue containing many small capillaries. Near the upper end of the tarsus, the layer of infiltrated inflammatory cells was thicker and contained plasma cells and histiocytes. The lids of the uninoculated eye showed a similar picture. The centers of the follicles contained groups of epithelioid cells.

AUTHOR'S SUMMARY.

FILTRABILITY AND POLYMORPHISM OF TUBERCLE BACILLI. A. FONTES, Beitr. z. Klin. d. Tuberk. **77**:2, 1931.

Intracellular chromatin granules of tubercle bacilli can propagate without the rest of the bacterial body. The granular form is a phase in the life cycle of the organism. The life cycle is represented essentially by the following phases: granular dust, free granules, multiplication of granules, cellular organization, production of intracellular granules, production of extracellular granules, propagation of bacterial cells. The granule is the living entity that can produce infection. Tuberculous virus may be present in the animal body without producing the

characteristic tissue reactions. The virulence is variable, as shown by its decrease after filtration. Scrofulosis and hereditary infections are due to a special phase of the virus.

MAX PINNER.

FILTRABLE FORMS OF TUBERCLE BACILLI. A. VAUDREMER, Beitr. z. Klin. d. Tuberk. **77**:16, 1931.

The tubercle bacillus is not always acid-fast. It possesses a true endotoxin. Tuberculin is an exogenous poison and can produce sensibilization. The tubercle bacillus is very variable. When it is grown at low temperature and with a minimum of nutrient material, atypical forms are developed. In the life cycle of the organism there occurs a stage at which the nuclear substance seems to be in an amorphous state. Between this amorphous state and the visible granules there is a stage in which the virus is filtrable.

MAX PINNER.

VARIABILITY OF THE TUBERCLE BACILLUS. S. S. MAHER, Beitr. z. Klin. d. Tuberk. **77**:40, 1931.

Atypical strains of tubercle bacilli can be produced by culturing tubercle bacilli over long periods of time in liquid mediums. Out of the sedimented bacterial mass one obtains nonacid-fast granulated rods that frequently produce pigment. Similar variations can be produced by letting the culture medium dry. A strain of acid-fast rods is demonstrated, which was derived from the acid-fast spores of a culture of Bacillus subtilis. It is the author's belief that bovine tubercle bacilli are descendants of B. subtilis.

MAX PINNER.

VARIOUS FORMS OF TUBERCLE BACILLI. R. PLA Y ARMENGOL, Beitr. z. Klin. d. Tuberk. **77**:47, 1931.

The author shows photomicrographs of various forms of tubercle bacilli. These include cocci of various sizes, long granulated filaments and streptococcus-like forms.

MAX-PINNER.

VARIATIONS OF THE TUBERCLE BACILLUS IN FORM AND ACTION. H. MUCH, Beitr. z. Klin. d. Tuberk. **77**:60, 1931.

The tubercle bacillus exists in essentially two forms, in that described by Koch and in that described by Much. The latter form is very variable; it may consist of thick spheres, dustlike granules or invisible forms. In the latter stage it is filtrable and may produce atypical types of infection, but the classic form can always be regenerated. Tubercle bacilli can be lysed by injecting them into growing plants. In some plants tubercle bacilli are completely lysed, so that they cannot be demonstrated microscopically or by infections of animals. In other plants tubercle bacilli die without being lysed. In other plants tubercle bacilli are neither lysed nor killed. And in still other plants tubercle bacilli remain alive, but lose their virulence.

MAX PINNER.

MORPHOLOGIC VARIATION OF TUBERCLE BACILLI. O. KIRCHNER, Beitr. z. Klin. d. Tuberk. **77**:72, 1931.

By growing tubercle bacilli submerged in liquid mediums atypical forms are obtained. The latter are shown in a colored illustration.

MAX PINNER.

THE DEVELOPMENTAL CYCLE OF THE TUBERCLE BACILLI. H. MOLLGAARD, Beitr. z. Klin. d. Tuberk. **77**:83, 1931.

If bovine or human tubercle bacilli are seeded on mediums containing filtered bone-marrow extract or autoclaved yeast extract, nonacid-fast and partly granu-

lated rods appear first. They are first gram-negative, but the granules, frequently in diplococci arrangement, become gram-positive. On prolonged culturing on these mediums, long filaments and sometimes typical actinomycetes with gonidia occur. The same forms were recovered from animals after infection. The same corynelike and actinomycotic forms were obtained from the bone-marrow of tuberculous calves, and the same forms were recovered from the blood of human beings with pulmonary tuberculosis in the acute stage. The diplococcic forms grow out to rods, which are either gram-negative or gram-positive or acid-fast. The gram-positive rods develop on Petroff's medium into typical acid-fast rods. It is important in repeating the studies to adjust the mediums to p_H 7.3 and to keep them in an atmosphere of 5 per cent carbon dioxide. The bone-marrow for the mediums must be taken from very young calves; the yeast extract must be prepared from dry yeast. The nonacid-fast forms occur usually between the fourteenth and twenty-first days. The culture medium remains clear and contains granular matter, which usually adheres to the wall and to the bottom of the tube. All nonacid-fast forms were nonmotile.

MAX PINNER.

Immunology

INTRAVENOUS VACCINATION WITH HEMOLYTIC STREPTOCOCCI. M. G. WILSON and H. F. SWIFT, Am. J. Dis. Child. **42**:42, 1931.

The seasonal incidence of the recurrence of rheumatic activity among 172 children of the susceptibile age group observed for a period of four years is presented. About half of these children received intravenous vaccination with hemolytic streptococcal vaccine. The remaining number were observed as a control group. The incidence of recurrence and manifestations of activity in both groups were comparable during the years 1927 and 1928, before vaccination was given. The yearly incidence of recurrence was less in the treated group than in the control group during the years 1929 and 1930, after treatment. Forty-five per cent of the treated children, as compared with 18 per cent of the controls, were free from recurrence for periods of from sixteen months to two years after treatment. The causal relation of intravenous vaccination with hemolytic streptococcal vaccine to the diminished incidence of recurrence observed is discussed.

AUTHORS' SUMMARY.

THE INFLUENCE OF DEHYDRATION ON ANAPHYLAXIS IN GUINEA PIGS. M. I. RUBIN and C. E. KELLETT, Bull. Johns Hopkins Hosp. **49**:170, 1931.

Dehydration is shown to protect a sensitized guinea-pig from anaphylactic shock. The experiments with histamine suggest that the protection thus afforded is in part, at any rate, due to diminished irritability of smooth muscle. We have pointed out that dehydration may explain the success of many of the other anti-anaphylactic procedures. The clinical application of the results of our experiments are encouraging.

AUTHORS' SUMMARY.

THE SKIN AS AN IMMUNOLOGICAL ORGAN. L. TUFT, J. Immunol. **21**:85, 1931.

During the course of some experiments in serum-sickness, it was observed that the skin seemed to possess an unusual capacity for sensitization to horse serum, this sensitization being accompanied by the production of anaphylactic antibodies, and these experiments suggesting the possibility that the skin may act as an important immunologic organ. Numerous clinical observations bear out the close relationship existing between the skin and processes of immunity in infectious and allergic diseases. These have been further substantiated by many experimental contributions reported in the past few years in the foreign literature, which appear to

indicate not only that the skin may have the power to form antibodies, but that such antibodies may be specific and that the ability to form such antibodies resides specificially in the skin. My own experiments do not bear out the latter view, at least for antibacterial antibodies, for from a comparison of the antibody response after the intradermal, subcutaneous, intramuscular and intravenous injection of mixed typhoid vaccine, both in the human being and in the lower animals, it would appear that though it is possible that the skin participates actively in antibody production, it does not carry out this function exclusively in respect to other tissues or organs. The marked antibody response particularly after local injection can be best explained on the basis of a local tissue stimulation of the cells of the reticulo-endothelial system, these cells being particularly abundant in the skin. That the active antibody response after intradermal injection may be due in part to a slower absorption of the antigen is entirely possible, although this process may in turn allow for local fixation of a greater amount of antigen and thus greater local stimulation of antibody.

AUTHOR'S SUMMARY.

DIPHTHERIA ANTITOXIN IN HUMAN SWEAT. J. M. NEILL, E. L. GASPARI, R. A. MOSLEY and J. Y. SUGG, J. Immunol. **21**:101, 1931.

This paper deals with diphtheria antitoxin in human sweat. Significant amounts were found in the sweat of three men possessing large amounts in their blood (two with "natural" immunity and one previously given an injection of toxoid); none was found in the sweat of people with only small amounts of antitoxin or none in their blood. The data are compared with previous data on saliva and urine on the basis of the following questions: the comparative frequency of persons with detectable antitoxin in the secretion, comparative ratios to serum antitoxin, daily output and significance of sweat as a channel of loss of immune substances.

AUTHORS' SUMMARY.

A QUANTITATIVE ASPECT OF THE HYPOTHETICAL INCORPORATION OF INJECTED ANTIGEN IN RESULTING ANTIBODY. S. B. HOOKER and W. C. BOYD, J. Immunol. **21**:113, 1931.

From quantitative data relative to the weight of injected antigen and to the potency of resulting antibody, it is conservatively estimated that one molecule of active antigenic substance gives rise to an amount of agglutinin capable of flocking 600 bacteria. This result is difficult to reconcile with the hypothesis that antibody is a conjugate of antigen and body globulin. The surface relationship (implicated in the mechanics of agglutination) between one globulin molecule and 600 bacteria is 1:25,000,000. Even with the assumption of multiple monospecific determinants in a single molecule of antigen this discrepancy is extreme. A theory of antibody formation involving catalysis would seem more promising.

AUTHORS' SUMMARY.

ON THE MECHANISM OF TOXIN-ANTITOXIN REACTIONS. J. FREUND, J. Immunol. **21**:127, 1931.

High dilutions of tannin in a neutral or slightly acid menstruum detoxify diphtheria and tetanus toxins. The detoxifying effect of tannin can be demonstrated on either highly diluted toxins or toxins absorbed on collodion particles. There is a characteristic prezone in the effect of tannin on toxin. Dilutions from 1:100 to 1:3,200 are not so effective as higher dilutions. (The same prezone characterizes the agglutination of red blood cells by tannin.) These experiments seem to be in harmony with the view that the various reactions demonstrated with a given antigen are all due to the same antibody and that the mechanism of all antigen-antibody reactions is the same.

AUTHOR'S SUMMARY.

AGGLUTININ PRODUCTION IN SUPRARENALECTOMIZED RATS. D. KHORAZO, J. Immunol. **21**:151, 1931.

In contrast to previous work, we have been unable to demonstrate that bilateral suprarenalectomy in rats alters agglutinin production against typhoid vaccine.

AUTHOR'S SUMMARY.

TOXINS OF STREPTOCOCCUS EPIDEMICUS. I. E. PILOT and I. DREYER, J. Infect. Dis. **49**:135, 1931.

Streptococcus epidemicus of epidemic septic sore throat produces a toxin, as determined by intradermal tests; 98 of 324 persons (30 per cent) giving positive reactions. Susceptible persons, as well as rabbits, may be immunized with the epidemicus toxin. Skin reactions to the toxins of S. epidemicus are widely divergent from the reactions to the toxin of S. scarlatinae. Of 208 persons, 46 gave positive reactions to scarlet fever toxin, 56 to the epidemicus toxin and only 10 to both toxins. An attack of scarlet fever resulted in a loss of the skin reaction to the scarlet fever toxin, but did not affect the skin reaction to the epidemicus toxin. Of 24 patients convalescent from scarlet fever who gave negative reactions to the Dick test, 8 (33 per cent) reacted to the epidemicus toxin. In persons giving positive reactions to both toxins, immunization with scarlet fever toxin resulted in the disappearance of the susceptibility to the scarlet fever toxin, but did not affect the susceptibility to the epidemicus toxin; immunization with the epidemicus toxin resulted in loss of susceptibility to this toxin, while the result of the Dick test remained positive. Antiserums produced by means of the epidemicus toxin appear to be specific. They neutralize the epidemicus toxin, but do not affect the scarlet fever toxin in persons giving positive reactions to skin tests with both toxins.

AUTHORS' SUMMARY.

THE SPECIFICITY OF AVIAN TUBERCULIN REACTIONS. V. B. DOLGOPOL, J. Infect. Dis. **49**:216, 1931.

A positive reaction to avian tuberculin, observed in many cases of pulmonary tuberculosis, does not indicate an active mixed infection with human and avian tubercle bacilli. (This conclusion is based on the result of the inoculation of chickens with the sputum of ten patients who showed positive reactions to avian tuberculin). A positive reaction to avian tuberculin in pulmonary tuberculosis does not indicate latent infection with the avian tubercle bacillus. (This conclusion is based on the result of the inoculation of chickens with autopsy material, including peribronchial lymph nodes, from three patients who had shown positive reactions with avian tuberculin). The large percentage of positive reactions with avian tuberculin in tuberculous patients, observed by Maggiore, by Stewart and Collins, by L'Esperance, and by myself, must be considered only as a large percentage of group reactions in patients suffering from tuberculosis caused by the mammalian types of tubercle bacilli. In the presence of positive cutaneous reactions with mammalian tuberculins a positive reaction with avian tuberculin cannot be taken as indicating an infection with the avian tubercle bacillus; but such an infection may be assumed to exist when the reaction to avian tuberculin is positive while the reaction to other tuberculins is negative or considerably weaker. Tuberculosis does not develop in chickens when they have been inoculated intravenously or intraperitoneally with large amounts of mammalian tubercle bacilli.

AUTHOR'S SUMMARY.

THE NATURE OF THE ANTIBODIES. PAUL VON GARA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:1, 1931.

Ninety rabbits divided into small groups were given injections of ox serum, Eberthella typhi or sheep red blood cells. A few were treated with only one

antigen, but most were also given injections of the other antigens, and their serums were then studied for agglutinins, precipitins, hemolysins, complement fixing and bactericidal properties. With the exception of the bactericidal properties, which decreased, all the other antibodies showed a parallel increase, depending on the number of injections, two of which were the minimum necessary to produce a response. Anamnestic reactions following a single repeated injection of a homologous or heterologous antigen were noticed even when the interval between the injections of the two antigens was considerable. Following multiple injections of the new antigen, such anamnestic reactions could not be seen. The response to reinjections after intervals was also greater than to first injections. Absorption with the homologous antigen removed the antibodies completely, while absorption with heterologous antigen, though marked, was not equally complete, though parallel for all antibodies. The author concludes that the so-called different antibodies are only different reactive forms of the condition of the serum resulting from the injection of the antigens.

I. DAVIDSOHN.

BACTERICIDAL SUBSTANCES IN TISSUE CULTURES. SHUJI KOMATSU, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:76, 1931.

The formation of bactericidal substances was observed in cultures of the spleen and to a lesser extent of the bone-marrow of rabbits given injections of Eberthella typhi. The introduction of the antigen into the culture of the tissue was not followed by the development of bactericidal properties. They appear in the tissues, particularly in the spleen, earlier than in the blood serum. The development of bactericidal substances in tissue cultures of nontreated animals could not be observed.

I. DAVIDSOHN.

THE OCCURRENCE OF THE SEROLOGIC FACTORS M AND N IN THE JAPANESE. S. SHIGENO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:88, 1931.

In 329 blood specimens sent from Japan to Berlin both factors were found in numbers similar to those observed in Europeans and whites of North America. A decrease in the titer, particularly of factor N, was observed in the transported blood specimens.

I. DAVIDSOHN.

THE SPECIFIC (IMMUNOLOGIC) PROPERTIES OF PURIFIED TOXINS. S. SCHMIDT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:101, 1931.

Dipththeria anatoxins from various sources were successfully purified by adsorption with aluminum hydroxide. A reversal of the original detoxifying process with a return of the toxicity was not observed as a result of purification and concentration. Occasional slight increases of toxicity were, in the author's opinion, due to a concentration of a small fraction which was not detoxified during the preparation of the anatoxin. Besides the procedure of Ramon, a test for toxicity is suggested, consisting in an intracutaneous injection of 0.2 cc. into a rabbit. Purified and concentrated toxins and anatoxins showed better antigenic properties than the crude products. Good results were obtained when the immunization was begun with anatoxin and continued with toxin.

I. DAVIDSOHN.

Tumors

NEUROGENIC SARCOMA. FRED W. STEWART and MURRAY M. COPELAND, Am. J. Cancer **15**:1235, 1931.

After a detailed study of the material of the Memorial Hospital the authors conclude that neurogenic sarcoma is an exceedingly variable disease. A large percentage of sarcomas of the soft parts are of neurogenic origin and possess the same gross and microscopic features and run the same clinical course as

so-called Recklinghausen's disease. The fundamental cell of origin of the tumor is the Schwann cell. The lamellar sheath contributes to certain of these tumors. Although they may occur anywhere, they have sites of predilection and a tendency to involve certain nerves. After excision they recur, as a rule. However, these recurrences are no other than new tumors arising from nerves in the vicinity. Evidence that irradiation after excision prevents or delays recurrence is lacking. The article contains a comprehensive discussion on the pathology of the tumors, and the reports of sixty-four selected cases of neurosarcoma from the literature in the form of tables. The references are abundant.

B. M. FRIED.

OLIGODENDROGLIOMA. S. T. KWAN and BERNARD J. ALPERS, Arch. Neurol. & Psychiat. **26**:279, 1931.

Kwan and Alpers give a detailed report of four cases of a type of a glioma classified by Cushing and Bailey as oligodendroglioma. They confirm the view that the latter is a specific, cellular and slowly growing tumor, consisting of glia or naked nuclei. These tumors are scattered in immense amounts in the subcortical white matter along the axons and are, of course, also present in the gray matter, mainly as satellites of the ganglion cells. With specific stains they show processes and are preferably designated as oligodendroglia or oligogliocytes. It is rather noteworthy that tumors should be made up of fully differentiated elements, as the oligodendroglioma is. Among oligogliocytes forming the latter, Kwan and Alpers also found unipolar and bipolar spongioblasts and transition forms between oligogliocytes and astrocytes. In some instances they were able to show the development of both oligogliocytes and the transition forms from the spongioblasts. An interesting feature was the presence, in one case, of tumor masses in the subarachnoid space, for which the authors, however, fail to give an adequate explanation.

GEORGE B. HASSIN.

MULTIPLE NEUROFIBROMATOSIS. K. HOSOI, Arch. Surg. **22**:258, 1931.

The author had been able to follow a benign neurofibroma through to a spindle cell sarcoma. Microscopic section of the lesion revealed a transformation from the relatively acellular neurofibroma to a cellular spindle cell tumor. Hosoi gives an extensive review of the literature. A total of sixty-five cases of sarcomatous transformation of neurofibromatosis have been collected. Seventy-two per cent of the cases occurred between the ages of 30 and 50. Malignant transformation takes place in about 13 per cent of all cases of Recklinghausen's disease. The tumor grows rapidly and tends to recur. Metastases are late. The prognosis is poor.

N. ENZER.

ADENOID CYSTIC CARCINOMA WITH GENERALIZED METASTASES IN THREE CASES OF THE BASAL CELL TYPE. A. W. SPIES, Arch. Surg. **21**:365, 1930.

The author reviews at considerable length the adenomatous types of basal cell tumor in the skin and mucosa, emphasizing the fact that true basal cell tumors of the mucosa are not universally accepted. The general conception that basal cell tumors are benign will have to be modified to the extent that basal cell tumors showing an adenoid structure may be malignant, as illustrated by three cases reported here. Spies has been unable to find an authentic, proved case of basal cell epithelioma with metastases. Most of those which have been reported as basal cell tumors with metastases were probably basal cell tumors with some squamous cell features. The adenoid cystic carcinomas of the skin are chronic, localized lesions, generally occurring at a younger age than the true basal cell carcinoma. They are radio-sensensitive. Those on the mucosa are much more malignant. These histologic features of both the cutaneous and noncutaneous groups are similar. The characteristic feature is the cordlike arrangement of cells of the basal type thrown into papillary and pseudoglandular formations. Occasional

solid alveolar forms and discrete cyst forms are found. Mitoses are infrequent. The formation of the cysts is open to some interpretation. Some authors believe that they are due to degeneration of the stroma. Others believe that they are results of secretory and desquamative activity of the cells themselves. In general, the adenoid structures of these tumors are poorly formed, and the unit is difficult to detect. The stroma is scanty.

N. ENZER.

CARCINOID TUMORS OF SMALL INTESTINE. H. H. COOKE, Arch. Surg. **22**:568, 1931.

Eleven cases are reported, three of which were malignant. The primary lesion was usually a circumscribed nodule. Multiple nodules were present in three cases. One of them formed an annular tumor. Nine of these were in the ileum and two in the jejunum. Metastases occurred in 20 per cent of reported cases, despite the fact that the histology of the carcinoid tumors generally suggests a benign tumor.

N. ENZER.

SARCOMA OF THE ESOPHAGUS. H. J. DVORAK, Arch. Surg. **22**:794, 1931.

Sarcoma of the esophagus is more frequent than carcinoma of the esophagus in young persons, its most frequent site being in the lower third. It may be a polypoid or solid medullary spindle cell tumor invading the wall. The author reports a case of rhabdomyosarcoma. About thirty cases have been reported in the literature.

N. ENZER.

INJURY AND GLIOMA OF BRAIN. H. L. PARKER and J. W. KERNOHAN, J. A. M. A. **97**:535, 1931.

A series of 431 cases of glioma of the brain have been studied in relation to injury to the head. Injury was reported to have occurred in 58 cases (13.4 per cent). As a control, in an equal number of cases without tumor of the brain there was a history of injury to the head in 45 (10.4 per cent). Two hundred normal persons were also interrogated, and 71 (35.5 per cent) reported injury to the head. With or without adjustment in patients with tumor for relatively greater liability to fall and be injured, the incidence of injury in this group is not sufficiently high, as compared to the other series, to suggest an etiologic factor. The relative incidence of injury in cases of glioma of the brain must therefore be abandoned as an argument in favor of injury being a cause of glioma. Considering the relatively high incidence of injuries to the head in the general population, there is ample possibility that the occurrence of the injury and the tumor in the same case may be mere coincidence.

Certain criteria are suggested to apply to a given case of glioma of the brain before the reported injury can even be assumed as a cause, especially in medicolegal cases. The 58 cases in which there was a claim of injury to the head subsequent to death or operation were found, in the light of these criteria, to shrink to 21, wherein, for the purpose of argument, injury might be assumed as cause. This represents only 4.2 per cent of the total series of gliomas of the brain. Therefore, in 95.8 per cent of the cases, injury had had played a part in the formation of tumor and accordingly cannot be a significant factor in the general causation of glioma. Further, in the cases considered here, both gross and microscopic examination of tissues failed to show any association between the tumor and an old injury.

Since the Great War, at least 2,858 cases of injury to the head, civil and military, have been reported in current literature. The patients have been observed for periods of from two months to twelve years. In none of the 2,858 cases was there a record of glioma of the brain developing as a result of these injuries. Therefore, glioma of the brain as a sequela of injury to the head must

be excessively rare and comes within the possibility of coincidence. The cause of glioma of the brain is still unknown, and as yet it is unwise to affirm that injury has any direct causal association, for the preponderance of evidence is against this conclusion.

AUTHORS' SUMMARY.

PRIMARY MALIGNANT ADAMANTINOMA OF THE OVARY. M. N. ZAJEWLOSCHIN, Frankfurt. Ztschr. f. Path. **41**:100, 1931.

A primary malignant adamantinoma of the ovary in a girl 8 years old is reported. During the operation for removal, it was noted that both tubes and the uterus were absent. The tumor weighed 200 Gm., and measured 6 by 7.5 by 9 cm. Its surface was in part smooth and in part nodular. On section, the bulk of the tumor consisted of an irregular lobular mass, a small amount of interstitial tissue and some calcified areas. A few cysts were also noted. Histologically, the tumor revealed typical adamantinoma structures. The author believes that the tumor reported is a congenital teratoma in which the predominant portions are represented by the adamantinoma. It is possible that originally this tumor was a dermoid cyst with tooth structures, from which the adamantinoma developed.

OTTO SAPHIR.

COLLISION TUMORS. P. GOETTING, Frankfurt. Ztschr. f. Path. **41**:107, 1931.

A pyloric tumor was removed from a man 38 years old. The liver, which was examined during the operation, showed no evidence of metastasis. A roentgen examination five months after operation revealed metastases in the lung. On palpation a metastatic tumor was also recognized in the liver. When the patient died subsequently, no autopsy could be obtained. Histologic examination of the pyloric tumor revealed a typical cylindric cell carcinoma and a spindle cell sarcoma which infiltrated the carcinoma. The various explanations of the histogenesis of these tumors are discussed.

OTTO SAPHIR.

CANCER IN HAMBURG. W. SCHWANKE, Ztschr. f. Krebsforsch. **32**:259, 1930.

Owing to the fact that immigrants to Hamburg consist almost entirely of young persons, the cancer statistics of that city permit of unusually close coordination as regards age incidence. There has been a great increase in late years of persons of advanced age. In 1900, 41 per cent of the population was over 30 years of age; in 1929, 53 per cent. During this period the general population has increased 1.6 times, while the incidence of cancer has risen 2.8 times. Under the age of 30 there has been no increase of cancer. The matter of age, however, is not held by Schwanke as the sole factor concerned in this increase, which he ascribes in part to the efficiency of modern prophylactic measures against general harmful agencies except such as contribute to malignancy. The rise in the cancer rate occurred not uniformly, but as successively higher waves. Despite the fact that the proportion of females in the population has increased, the relative incidence of cancer cases for this sex has decreased from 58 per cent for the period from 1872 to 1899 to 55 per cent for the period from 1902 to 1929. The statistics showed nothing in the way of a definitely reciprocal relationship between cancer and tuberculosis. The distribution by organs was as follows: In males 79 per cent, and in females, 54 per cent, were located in the alimentary tract; some decrease in cancer of the female genital organs was more than balanced by an increase of cancer in other organs, especially the breast. Even so, cancer of the aggregate sex organs was less frequent in the female sex than alimentary cancer, a shift in distribution that appears to have been in evidence ever since 1872. In males, on the other hand, there has been a slight diminution in the incidence of alimentary cancer. It would appear that diminished occurrence of cancer in one group of organs is associated with an increase elsewhere in the body.

H. E. EGGERS.

ADAMANTINOMATOUS TUMOR OF THE TIBIA. C. S. RICHTER, Ztschr. f. Krebsforsch. **32**:273, 1930.

There is here reported a tibial tumor of a Javanese in which the microscopic appearances were those of adamantinoma. This is the second case of the sort to be reported. The author regards it as a peculiarly differentiated bone endothelioma.

H. E. EGGERS.

Medicolegal Pathology

COLLODION AS AN INSTRUMENT OF SUICIDE. M. MOSKOW, Ann. de méd. lég. **11**:78, 1931.

The lungs of a suicide were found to contain hardened collodion ramified throughout the bronchial tree. That the liquid evidently acted not as a poison but as a mechanical agent producing suffocation was demonstrated in an experimental study in which collodion was poured into the mouths of animals. In the majority, choking was followed by aspiration of the liquid and death, and hardened collodion was found in the respiratory passages. The suicide was complicated by the attempt of the victim to strangle himself with a strap, apparently in order to relieve the pain caused by penetration of the collodion into the lungs.

E. M. BARTON.

STATUS THYMICOLYMPHATICUS IN LEGAL MEDICINE. W. G. DABROWSKI, Ann. de méd. lég. **11**:128, 1931.

Studies were made of thymic and lymphoid tissue from 920 bodies. When death occurred quickly, whether due to suicide, to other violence or to fulminating disease, the thymus gland and lymphatic tissue were well developed in most young undebilitated persons, and no great difference in the development of those tissues was found in subjects of the same age group. A persistent thymus or so-called status thymicolymphaticus, therefore, cannot be considered proof of a predisposition to sudden death. Indeed, in these observations, proof of status thymicolymphaticus as an innate constitutional characteristic was lacking. Overdeveloped thymic and lymphatic tissue was not found more frequently in suicides than in other bodies when death had occurred quickly.

E. M. BARTON.

TRAUMATIC APPENDICITIS. P. WIART, Ann. de méd. lég. **11**:381, 1931.

True traumatic appendicitis is extremely rare. The lesions must be shown to be inflammation secondary to mild trauma in a hitherto normal organ. If traumatization is severe, the condition anatomatically becomes classified with other abdominal contusions, whether the appendix was previously normal or diseased. All clinical manifestations and sequelae of these conditions should be attributed to the accident. When symptoms of appendicitis first appear at a time considerably removed from the accident the responsibility of trauma, provided there was a previously normal appendix, can rarely be established. In industrial accidents involving chronically inflamed appendexes only the immediate consequences of the accident can be admitted, while in "common law" accidents, the responsibility must be divided pro rata between the previous chronic state and trauma that conceivably might leave a normal appendix uninjured.

E. M. BARTON.

BLOODY SPINAL FLUID FOLLOWING AN OSTEOPATHIC TREATMENT. M. TRÉUEL, Ann. de méd. lég. **11**:488, 1931.

Several weeks after a "painful and violent" osteopathic treatment a woman, 34 years old, with a family history of syphilis and a negative Wassermann reaction of the blood had a violent attack of mania. In xanthochromatic spinal fluid

under increased pressure there was blood, albumin, a positive Wassermann reaction and bizarre changes in the colloidal gold curve. Three weeks later the cerebrospinal fluid was essentially normal. The author believes that the bloody fluid was of purely traumatic origin, due to the osteopathic manipulations.

E. M. BARTON.

APOPLEXY OR SUICIDE? FRITZ ARNDTS, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:164, 1931.

The precarious economic conditions in Germany, which have now extended over a long period of time, have led to many fraudulent actions against the insurance companies. Persons whose financial status is close to ruin take out high life insurance policies with accident indemnity, in order to protect their families financially, and, shortly afterward, under inexplicable or mysterious circumstances, their sudden death is reported. A pertinent case of this kind is presented in a thorough critical analysis, and it is recommended that in every similar instance an autopsy by competent medicolegal pathologists should be performed in order to clear the situation, with particular view in mind of a possible poisoning with modern hypnotics.

E. L. MILOSLAVICH.

MECHANISM OF SUDDEN DEATH FOLLOWING ACUTE CIRCULATORY DISTURBANCES. ERICH BRACK, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:176, 1931.

In instances of death due to a massive, suddenly developing hemorrhage, there is a contraction not only of both ventricles of the heart but also of large arterial vessels, particularly of the aorta. Thus, the blood is forced into the peripheral parts of the body, into the body cavities (internal hemorrhage) or to the outside (external hemorrhage), rapidly causing death. The parenchymatous organs do not show any marked signs of anemia, such as are constantly observed in cases of slowly occurring bleeding in the gastro-intestinal canal or from the female genital tract. Minute injuries to the walls of the heart, especially to the ventricles, or to the large arteries, such as the aorta, carotids, etc., may not cause any alarming symptoms and may gradually heal up. The simultaneously traumatized perivascular sympathetic plexus contracts the injured vessel, giving it a chance to cover the area with thrombotic material. Persons suffering with hypertension usually show profuse uncontrollable hemorrhages due to lack of the normal vasoconstrictive regulation, and the internal organs present higher degrees of loss of blood, as in instances of ordinary acute hemorrhages. Organs, such as the kidney, liver or spleen, may show a normal amount of blood if a topographically distant artery is severed. An abundant loss of blood (up to 2 liters) is endured far more easily by females than by males. The sudden constriction of the arterial system, as observed in various traumatic incidents, displaces the blood volume into the veins, and all the internal organs present a deeply cyanotic appearance, contributing to the suddenness of death. A similar mechanism and conditions are met with in cases of asphyxia due to mechanical obstruction of the upper respiratory tract. The rapidly developing increase of blood volume (plethora), as in acute alcoholism, is evidenced at autopsy by a dilatation of the entire arterial tree, and the left ventricle of the heart is dilated while the right ventricle is rather contracted and the cavae collapsed. In certain cases of fatal hemorrhage due to injury, one finds an extremely pale skin and testicles, while the internal organs exhibit a marked congestion. This abnormal blood supply is undoubtedly the effect of vasomotor action. Changes in the left branch of the conducting system of the heart, which often are difficult to establish, cause sudden death from a severe attack of the Adams-Stokes' type. Cor bovinum, which results from a miliary myocardial fibrosis, leads to unsuspected death because of an excessive, rapidly occurring blood supply to the myocardium. The so-called aorta angusta,

as found in certain cases of acute lethal circulatory disturbance, is the result of a vasomotor contraction. In a certain group of cases, diffuse dilatations of peripheral arteries, as those at the base of the brain or in the kidneys and heart, constitute the only anatomically detectable evidence of acute circulatory distress. Microscopic thrombosis of various organs, particularly of the brain, as seen in various poisonings, certainly forms the anatomic basis of sudden death. Pronounced congestion of the suprarenals at their corticomedullary boundary hinders the function and obstructs the transportation of the secretions of these vital organs, leading to their acute insufficiency.

E. L. MILOSLAVICH.

MEDICOLEGAL IMPORTANCE OF CERTAIN NEW ELECTROPATHOLOGIC FINDINGS. A. D. KAPLAN, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:217, 1931.

In the course of an electrical current of high tension, between the entrance and exit, one may find typical cutaneous burns on the surface of the body, particularly if the skin was folded or the extremities bent, as in the groin or armpit, such burns may indicate the position of the body at the time of accident. The spindle-like appearance of the cells of the malpighian layer is not characteristic of an electric mark, as similar changes are observed in ordinary burns, as, for instance, in those produced by a cautery. But changes of the various tissue structures beneath the epidermis, such as a marked elongation of the epithelial elements (protoplasm and nuclei) of the sweat glands, elongation of the endothelial cells of the capillaries and tears of blood vessels with perivascular hematoma, in connection with changes of the malpighian layer, are the result only of an electrical burn. The development of irregularly (zigzag) formed, tearlike channels in the hyalinized connective tissue of the cutis is of particular significance, as such formations do not occur in ordinary burns.

E. L. MILOSLAVICH.

COMPLICATIONS FOLLOWING ENDOCARDIAC INJECTIONS. WALDEMAR WEIMANN, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:244, 1931.

Injections of epinephrine into the heart may lead to some unexpected complications. There are instances in which the internal mammary artery was punctured and a severe hemorrhage followed, or in which the lung was pierced and a pneumothorax developed. In the case described, subepicardial branches of the left coronary artery were punctured by repeated insertions of the needle, with the subsequent development of a hemopericardium.

E. L. MILOSLAVICH.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

Oct. 8, 1931

BALDUIN LUCKÉ, *President, in the Chair*

SOME PROBLEMS OF BACTERIAL RESPIRATION. MARJORY STEPHENSON, Biochemical Department, Cambridge University, England.

The fundamental problem which the anaerobic cell has to solve is that of finding an efficient substitute for oxidation by molecular oxygen as an energy-delivering process. Several mechanisms have been adopted for this purpose:

1. The exothermic fission of the hexose molecule into two three-carbon compounds. These subsequently rearrange themselves into stable compounds of lower energy content than the original hexose. The familiar alcoholic and lactic fermentations are examples of such anaerobic mechanisms for delivering energy:

$$C_6H_{12}O_6 = 2C_3H_6O_3 + 22.5 \text{ Kinetic Calories.}$$
$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 22.0 \text{ K. Cals.}$$

Both are greatly inferior quantitatively to the oxidative mechanism:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 674 \text{ K. Cals.}$$

2. Other mechanisms consisting essentially in replacing oxygen by some other substance to act as a hydrogen acceptor in the oxidative process. Thus lactic acid may be reduced to pyruvic acid either by the aerobic process of the reduction of oxygen to water or by the anaerobic processes of the reduction of nitrate to nitrite. Other substances capable of replacing oxygen are: fumaric acid by reduction to succinic; aspartic acid by deamination to fumaric and subsequent reduction; glycerol by reduction to dimethylene glycol, and carbon dioxide by reduction to methane. The ability to use these very various substances as oxidizing agents depends on specific activating enzymes, the possession of one or more of which enables various bacterial species to function as anaerobes.

Strict aerobes, such, for example, as the timothy bacillus, possess none of these enzymes, but have, on the other hand, a very complete battery of mechanisms activating molecular oxygen. In accordance with this observation, it was shown by a series of quantitative experiments that the timothy bacillus oxidizes its substrate as completely as a typical aerobic animal.

Regular Meeting, Oct. 30, 1931

BALDUIN LUCKÉ, *President, in the Chair*

THE ANNUAL GROSS LECTURE: GENERALIZED OSTEITIS FIBROSA AND ITS RELATION TO OSTEOMALACIA AND RICKETS. F. J. LANG, Professor of Pathology and Director of the Pathological Anatomical Institute of the University, Innsbruck, Austria.

Recent views in regard to the nature of osteitis fibrosa were discussed, especially the work of Pommer, who attributes the fibrosis of the bone marrow to the result of localized congestion and irritation and considers the cysts as results of hemorrhages.

The lecturer made extensive studies of the relationship between osteomalacia, rickets and osteitis fibrosa. He considered that parts of the skeleton subjected especially to strain become bent because of insufficient calcification. Owing to the peculiar structure and circulatory system of bone, permanent congestion of blood and lymph vessels result. The congestion and mechanical irritation then lead to osteitis fibrosa. The fibrosis of the bone marrow and formation of bone are secondary to circulatory disturbances that follow functional mechanical trauma in insufficiently calcified and softened bone.

In experimental and clinical studies of rickets, scurvy and osteomalacia, the lecturer regularly found osteitis fibrosa. Localized osteofibrotic changes are also observed in inflammatory processes, as in odontogenetic osteitis fibrosa of the jaw, in the immediate vicinity of metastases to bones, in callus formation and pseudarthrosis, in gout, and near tuberculous and syphilitic lesions of bone. This proves the secondary nature of osteitis fibrosa. In order to establish the basic nature of the disease, it was necessary to use exacting histologic methods showing both calcified and noncalcified bone, by means of many large sections from various bones, seeking the primary changes unobscured by fibrosis. Without such careful methods, incorrect diagnosis of primary osteitis fibrosa will be made.

The peculiar structure of bone and its circulatory system permit only a slight compensatory adaptation to circulatory disturbances. This and the irritation caused by the use of the bone under the given conditions are largely responsible for the origin of osteitis fibrosa. The functional point of view has proved to be of importance in explaining the relation between osteitis fibrosa, osteomalacia and rickets.

Book Reviews

Pathologie und Klinik in Einzeldarstellungen. Herausgegeben von L. Aschoff, H. Elias, H. Eppinger, C. Sternberg und K. F. Wenckebach. Band III. **Die Lebensvorgänge im normalen Knorpel und seine Wucherung bei Akromegalie.** Von Professor Dr. J. Erdheim, a. ö. Professor an der Universität Wien. Paper. Price, 18 marks. Pp. 160, with 31 illustrations. Berlin: Julius Springer, 1931.

The monograph is divided into four chapters. The first discusses the progressive changes during advancing age in the normal costal cartilage. The mode of origin and the histologic structure of the three cortical layers and of the nucleus of the costal cartilages are described. The degenerative changes appearing with advancing age are detailed, and the influence of these on the changes in the elasticity of the cartilage is coordinated. The healing and restoration that follow degeneration, especially in the nucleus of the cartilage, are also detailed. The chapter ends with a description of the costochondral junction, of the regressive changes occurring at the junctions, of the cement lines, of chondrophyte formation and, finally, the changes appearing in the cartilages during calcification.

The second chapter relates the changes found in the costal cartilages of a woman 71 years of age who suffered from clinical acromegalia for four years, and in whom at autopsy a small tumor was found in the anterior lobe of the pituitary body. The gland weighed 2.35 Gm. Death was due to apoplexy and bronchopneumonia. The bones were normal.

The cartilages, especially the costal, showed what Erdheim designates as the specific changes of acromegalia. This consists of enlargement of the second cortical layer due to hypertrophy and hyperplasia of the cells and an increase of the basophilic ground substance. This enlarged, thick layer appears white in the gross. On the contrary, in normal cartilages the second cortical layer is thin and yellow. The cartilage growth begins at the costochondral junction. Here it is most extensive, and a rosary results. The thickness of the cartilage diminishes as the sternum is approached. Erdheim indicates that in young adults with acromegalia proliferation of the costal cartilages may be much more extensive and much more uniform than in the case being described, and that other cartilages (epiphyseal) may participate in the general growth process.

The third cortical layer and the nucleus do not participate in the hypertrophic and hyperplastic process in advanced life, because they undergo degeneration. In the rib cartilages of patients with acromegalia numerous small and large spaces appear in these zones. The cavities result from resorption by tissue fluids and are most numerous near the costochondral junctions. Many become filled with connective tissue, but they may also contain cartilage débris. On the other hand, the connective tissue contained within these spaces may undergo cartilaginous transformation. Marrow may extend from the bony portion of the rib into the degenerated cartilage nucleus, and free hemorrhages may be present. Of the changes described, the one most stressed is proliferation and hypertrophy of the second cortical layer.

The third chapter deals with acromegalic changes in the joint cartilages. Erdheim also found specific changes, and he distinguishes them from those observed in primary arthritis deformans. They consist of hyperplasia and hypertrophy of the cells of the deeper portion of the articular cartilage. It is similar to the rib in that the proliferating cartilage is at some distance from the perichondrium. These changes are therefore distinguished from primary arthritis deformans, in which the cartilage proliferation begins on the surface. In acromegalia ulceration of the articular cartilage may eventually occur, but it progresses from the deeper

portion toward the surface. In this way the changes again differ from those of primary arthritis deformans, for in this condition the ulceration is from the surface downward. In addition, Erdheim points out that even when the joint cartilage changes of acromegalia are advanced, blood vessels do not penetrate the calcium-free cartilage, while in primary arthritis deformans vessels enter the calcium-free cartilage from the very beginning. The joints both in primary arthritis deformans and in acromegalia have peripheral exostoses.

The fourth chapter deals with the clinical application of the anatomic findings. In a few pages there is summarized much that is known concerning the relation of the pituitary to bone growth, especially in regard to dwarfism. Erdheim points out that a pituitary destruction at a very early age will lead to dwarfism and a delay in closure of the epiphyseal cartilage plates. Pituitary hypersecretion leads to prolonged endochondral ossification. He restates the recent view that the pituitary factor may be more important than the thyroid factor in the pathogenesis of cretinism. He speculates that since the pituitary stimulates cartilage growth, it may affect metabolism (he apparently means sulphur metabolism). He suggests that the surgeon consider the use of anterior pituitary preparations to hasten the union of fractures or in the treatment for delayed union. (The reviewer doubts very much whether the present pituitary preparations are sufficiently satisfactory to test this point. The local factor in delayed union is beyond question.) He advises physiologists and experimental pathologists to investigate the cartilages when studying the tissue changes following pituitary experiments. He suggests that there may be a pituitary factor in chondrodystrophy in which there is arrest of cartilage growth. Erdheim stretches the point when he tries to associate the cartilaginous metastases in the lungs of a patient with acromegalia suffering from an osteochondroma of the femur with pituitary hypersecretion.

The monograph bears the stamp of Professor Erdheim's knowledge of the pathologic anatomy of bone. This is a subject in which he has been continuously interested since his significant work on the relation of the parathyroids to rickets. Figures 2 and 19 show discrepancies between the legends and the markings of the photographs. The monograph also suffers from repetition. The book is recommended for those interested in pituitary disease and in the pathologic anatomy and physiology of bone.

Anatomie pathologique. By Maurice Letulle, professeur honoraire a la faculté de médecine de Paris, médecin honoraire de l'hôpital Boucicaut. Avec la collaboration de L. Nattan-Larrier, professeur au collége de France et A. Jacquelin, médecin des hôpitaux de Paris; L. Duclos, ophthalmologiste adjoint de l'hôpital chirurgical Gouin. E.-P. Normand, conservateur du musée d'anatomie pathologique générale Maurice-Letulle (hôpital Boucicaut). Three volumes. Price, 520 francs. Pp. 2,346, with 843 figures. Paris: Masson & Cie, 1931.

This is a posthumous work. The manuscript was completed by Maurice Letulle a few weeks before his death. In response to his expressed wish, it is published without any changes. In the preface by the author's son, Raymond Letulle, he states that the section on the mammary gland has been added because no such section was found among his father's papers. The chapters on the blood and on the visual apparatus were prepared in collaboration with A. Jacquelin and L. Duclos, respectively.

The work comes in three volumes, each containing more than seven hundred pages numbered consecutively, with an alphabetical index and an analytic table of contents at the end of the third volume. In neither the index nor the table is there any mention of the volume in which any particular page occurs, and the pages in each volume are not given on its back, a defect easily remedied, however, by marking the pages in each volume on the back. The binding is not substantial. The type is clear and easy to read, and the pages are pleasant to the eye. The illustrations, 843 in all, all in black and white, mostly drawings, all original, made

and arranged with taste and care, are of high and uniform excellence, in some cases perhaps a little too diagrammatic, but taken all in all of superior instructional value.

In the text as well as in footnotes reference is made frequently to other writers whose names are given, but without mentioning the place and time of publication, thus saving space and labor without much serious loss in view of the extent to which the medical literature now has been indexed in readily available forms. Only a very few American writers are quoted (blastomycosis is not mentioned).

The text is divided into a general part, devoted mainly to inflammatory processes and tumors. Then come sections on the circulatory and respiratory apparatus, the blood and blood-making organs, the digestive organs, the urogenital organs, the endocrine gland, the nervous system and the visual apparatus. The skin, the ear and the locomotive apparatus do not receive separate consideration. The section on the nervous system is incomplete; epidemic poliomyelitis, encephalitis, herpes, syringomyelia, etc., are not discussed.

The discussion is confined strictly to the gross and microscopic morphology of morbid lesions. The descriptions are clear, graphic and orderly. The lesions of syphilis and of tuberculosis are described excellently. The treatment of the lesions of syphilis in descriptive text and by illustrations merits special commendation. The illustrations of buccopharyngolaryngeal sporotrichosis are striking. As would be expected, the fungus of sporotrichosis is named erroneously Sporotrichum beurmanni in place of Sporotrichum schenckii. Curiously, there are no illustrations of the cells of the blood, and the recent developments in the cytology of leukocytes, endothelial cells and wandering cells are not considered. The reticulo-endothelial system is not considered as such. The tumors in general, and particularly the melanomas, are well described and illustrated.

Letulle's "Anatomie pathologique" is an interesting and valuable book. It gives a good picture of the French teachings in its field, and certainly merits a place in medical libraries.

Hieronymi Fracastorii de Contagione, et Contagiosis Morbis et eorum Curatione, Libri III. Translation and Notes by Wilmer Cave Wright, Ph.D., Professor of Greek in Bryn Mawr College. Cloth. Price, $4.50. Pp. 356. New York: G. P. Putnam's Sons, 1930.

Fracastorius, 1478-1553, is known best as the author of the famous poem "Syphilis," which was published in its final form in 1530 and in which he treats of the disease that since has borne the name he gave the poem. Numerous editions in Latin and several translations of the poem have appeared. A far more important work by Fracastorius is his prose treatise "De Contagione, et Contagione Morbis et eorum Curatione," published in 1546 and now for the first time translated into English. The translation is published as the second volume of the History of Medicine Series issued under the sponsorship of the Library of the New York Academy of Medicine.

In this book infectious diseases are considered for the first time under three groupings, namely, infections by direct contact, infections carried by intermediate agents, or formites as Fracastorius named them, and infections that come through the air from a distance. The discussion is carried on in terms that suggest a truly remarkable prevision of the discoveries in microbiology more than three hundred years later. Typhus is recognized as a distinct disease, and other diseases (syphilis, tuberculosis of the lungs, plague, rabies, exanthems) are considered. The translation and notes are the product of mature and thorough scholarship. The introduction gives a most interesting account of the life of Fracastorius, and the bibliography lists the important editions and translations of his books, as well as biographies and pertinent general works. A great medical classic has received a worthy English setting.

Books Received

CHEMICAL METHODS IN CLINICAL MEDICINE. By G. A. Harrison, B.A., M.D., B.Ch., M.R.C.S., L.R.C.P., Reader in Chemical Pathology in the University of London; Reader and Lecturer on Chemical Pathology in St. Bartholomew's Medical College. Cloth. Price, $5.25. Pp. 534. New York: The Macmillan Company, 1930.

VERHANDLUNGEN DER DEUTSCHEN PATHOLOGISCHEN GESELLSCHAFT. Im Auftrage des Vorständes herausgegeben von dem derzeitigen Schriftführer G. Schmorl in Dresden. Sechsundzwanzigste Tagung gehalten in München am 9.-11. April, 1931. Pp. 423. Mit 193 Abbildungen im Text und 11 Tafeln. Jena: Gustav Fischer, 1931.

THE QUANTITATIVE ESTIMATION OF VITAMIN D BY RADIOGRAPHY. By R. B. Bourdillon, H. M. Bruce, C. Fischmann and T. A. Webster. Medical Research Council, Special Report Series, No. 158. Price, 6 pence, net. Pp. 46. London: His Majesty's Stationery Office, 1931.

THE INFLUENCE OF DIET ON CARIES IN CHILDREN'S TEETH (INTERIM REPORT). By the Committee upon Dental Disease. Medical Research Council, Special Report Series, No. 159. Price, 6 pence, net. Pp. 19. London: His Majesty's Stationery Office, 1931.

SURGICAL PATHOLOGY OF THE SKIN, FASCIA, MUSCLES, TENDONS, BLOOD AND LYMPH VESSELS. By Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kansas, and Professor of Surgery, University of Kansas. Price, $5. Pp. 301, with 260 illustrations. Philadelphia: J. B. Lippincott Company, 1931.

A SYSTEM OF BACTERIOLOGY IN RELATION TO MEDICINE. Volume 9. Medical Research Council. Price, per volume, 1 pound, 1 shilling, net. London: His Majesty's Stationery Office, 1931. (It may be obtained from the British Library of Information, 5 East Forty-Fifth Street, New York.)

EIN BEWEGTES GELEHRTENLEBEN. ERINNERUNGEN UND ERLEBNISSE, KÄMPFE UND GEDANKEN. Von Otto Lubarsch. Pp. 606. Berlin: Julius Springer, 1931.

HIERONYMUS FRACASTORIUS. CONTAGION, CONTAGIOUS DISEASES AND THEIR TREATMENT. Translation and Notes by Wilmer Cave Wright, Ph.D., Professor of Greek in Bryn Mawr College. Pp. 356. New York: G. P. Putnam's Sons, 1930.

GRUNDRISS DER ENTWICKLUNG DES MENSCHEN. Von Dr. Alfred Fischel, o. Professor der Embryologie und Vorstand des Embryologischen Institutes der Wiener Universität. Paper. Price, 11 marks. Pp. 141, with 117 illustrations. Berlin: Julius Springer, 1931.

REPORT OF COMMITTEE ON STANDARD PRACTICES IN THE PROBLEM OF COMPENSATION OF OCCUPATIONAL DISEASES OF THE INDUSTRIAL HYGIENE SECTION, AMERICAN PUBLIC HEALTH ASSOCIATION. By Henry H. Kessler, M.D. (Chairman), Bernard S. Coleman, Emery R. Hayhurst, M.D., Ceorge M. Price, M.D., May R. Mayers, M.D., Elizabeth B. Bricker, M.D., R. R. Sayers, M.D., and Eleanor Rantoul. Paper. Price, $1.50. Pp. 124. New York: American Public Health Association, 1931.

LES EXTRAITS PANCRÉATIQUES DÉSINSULINÉS EN THÉRAPEUTIQUE. Par R. Giroux et N. Kisthinios, assistants à la clinique thérapeutique de l'Hôpital de la Pitie. Préface du Professeur H. Vaquez. Paper. Price, 16 francs. Pp. 126, with 9 illustrations. Paris: Masson & Cie, 1931.

INDEX TO VOLUME 12

The asterisk (*) preceding the page number indicates an original article in the Archives. Author entries are made for original articles and society transactions. Subject entries are made for all articles. Book Reviews, Obituaries and Society Transactions are indexed under these headings in their alphabetical order under the letters B, O, and S, respectively.

Abnormalities: See Anomalies and Deformities; and under names of organs and regions
Abortion, criminal, 503
Abscess, sporadic cellulitis and abscess due to streptococcus epidemicus, 1031
Acetylphenylhydrazine, nature of erythrocytic damage produced by acetylphenylhydrazine in vivo and in vitro, 326
Acid, lactic acid in blood in children, 647
  lactic acid in blood of newborn, 649
  nucleic, color reactions of, 484
  oxalic acid in blood, 1022
  oxidation of fatty acids of serum in anemia, 126
  silicic acid content of blood in tuberculous patients, significance of, 843
Acid-base equilibrium, hydrogen-ion concentration and acid-base equilibrium in normal pregnancy, 482
Acrocyanosis: See Cyanosis
Actinomycosis, etiology and pathogenesis, 844
  generalized actinomycosis (pyemic form), report of a case, *397
  pulmonary "pseudo-actinomycosis" (chromoblastomycosis?) in a capybara due to a hyphomycete producing ray fungi in tissue, 326
  starting as appendicitis with extensive visceral involvement, 839
Adamantinoma, primary malignant adamantinoma of ovary, 1041
  primary, of tibia, 501, 1042
Adenocarcinoma, action of magnesium on, of rat, 309
  quadruple inoculations of, 501
Adenoma, hyperinsulinism from B-cell adenoma of pancreas, 282
  intestinal, in swine, 500
Adrenals: See Suprarenals
Agglutination, autohemagglutination, 657
  isohemagglutination: work of Jan Janský with a critical analysis, 130
Agglutinins, experimental studies on formation of agglutinins and precipitins, 659
  group-specific human hemagglutinins, 300
  production in suprarenalectomized rats, 1037
Agranulocytosis: See Angina
Alcohol, detection at autopsy, 314
  in blood and brain, determination of, 664
Alkalosis and carbohydrate tolerance in malignant disease, 308
Allergy: See Anaphylaxis and Allergy
Amebiasis, comparative study of amebiasis in man, monkeys and cats, with special reference to formation of early lesions, 327
Ampulla: See Vater's ampulla
Amyloid bodies, histogenesis of amyloid bodies in central nervous system; rôle of microglia in formation of amyloid body, *229
Amyotoma: See Myatonia
Anaphylaxis and Allergy; anaphylactic and tuberculin types of hypersensitiveness, 299, 492
  anaphylactic shock from intracerebral injection of antigen, 844
  anaphylactogenic action of protein from filtrates of acid-fast bacteria, 299
  congenital protein hypersensitiveness in 2 generations, 298
  hypersensitiveness to soluble specific substances from yeast-like fungi, 470
Anaphylaxis—Continued
  influence of dehydration on anaphylaxis in guinea pigs, 1035
  local organ hypersensitiveness (rabbit's eye), 655
  organ metabolism by tissue activity and anaphylaxis, 638
  pulmonary permeability in dogs in relation to anaphylactic shock, 130
  skin as an immunological organ, 1035
  transmission of sensitization from mother to child, 494
Anderson, R. M.: Experimental pathology of liver; restoration of liver of white rat following partial surgical removal, *186
Anemia, Bartonella anemia in nonsplenectomized rats, *405
  Bartonella muris, 1026
  Bartonella muris; compensatory phenomena following splenectomy in adult albino rat, 676
  Bartonella muris; pathogenic changes during acute anemia, 675
  catalase action and glutathione content of erythrocytes in, 649
  cerebral, functional disturbances in central nervous system in, 832
  erythroleukosis and anemias, of fowl, *1
  experimental, relation of sulphur to, 140
  grave anemia in pregnant women from benzene intoxication, 310
  oxidation of fatty acids of serum in, 126
  pernicious, "acid deficit" in, 634
  pernicious, effect of liver treatment on anatomic changes in, 125
  splenic, (Gaucher's disease), 146
  splenic, pathologic anatomy of Gaucher's disease, 1020
Aneurysm, ruptured, of ductus arteriosus, 835
Angina; agranulocytosis, 289
  anatomic changes in agranulocytosis, 838
  bone marrow in agranulocytosis, 142
Angioneuromyoma, 852
Aniline, late complications of poisoning by aniline or similar substances, 311
Anomalies and deformities; asymmetry of head and face, 475
  syndrome characterized by congenital clouding of cornea and other anomalies, 470
Anthrax infection, tissue specificity in, 486
  spores of bacillus anthracis, 1030
Antibodies: See Antigens and Antibodies
Antigens and Antibodies; extirpation of antigenic depot and antibody production, 300
  absorption experiments with hemolytic antibodies, 497
  antibodies in urine after injection of antitoxic globulin, 298
  immunologic specificity of antigens of brain, 146
  nature of antibodies, 1037
  quantitative aspect of hypothetical incorporation of injected antigen in resulting antibody, 1036
  separation of hemolytic antibodies fixed by kaolin and their amboceptor function, 498
  specific antigens and antibodies of brain, 497
Antitoxin, surface phenomena in reaction of toxin and antitoxin, 495
  transmission to third generation, 300
Aortas, lipoid deposits in aortas of infants, 645
Aortitis, pathogenesis of perforating purulent aortitis and its sequelae, 124
  thrombotica, 836
Apoplexy: See Brain, hemorrhage
Apparatus, new brain slicer, *90
Appendicitis, actinomycosis starting as appendicitis with extensive visceral involvement, 839
  traumatic, 1042

Appendix, demonstration of perforation of, *598
method of cutting and sectioning, *598
method for examining, *598
Aqueduct of Sylvius: See under Brain
Arecallium hydrobromicum, fatal poisoning with, 664
Argentaffine, morphology of so-called argentaffine cells in pancreas, 1019
Arnold, L.: Menstruation and focal reactions, 141
Arsenic, stimulating effect of arsenic on chicken sarcoma, 502
Arsphenamine, rôle of diet in protecting against liver injury by, 830
Arteries: See also Aneurysm; Aorta; Arteriosclerosis; Blood pressure; Embolism; Thrombosis; etc.
anomalous origin and course of left coronary artery, *586
changes of arteries in increasing age, 1020
coronary, abnormal origin of left coronary artery, 124
direct communications between arteries and vein in rabbit's ear, 285
experimental arteritis and arteriosclerosis associated with streptococcal inoculations, *924
isolated giant growth of branch of pulmonary artery associated with congenital bronchiectasis, report of case, *387
pulmonary, progressive thrombosis of, 477
Arteriosclerosis, experimental arteritis and arteriosclerosis associated with streptococcal inoculation, *924
hypervitaminosis D and arteriosclerosis, *941
pulmonary, obliterative, 478
Arthritis deformans, assimilation of atlas and compression of medulla: clinical significance and pathology of torticollis and localized chronic arthritis deformans of spine; report of a case, *333
Asbestosis bodies in feces in pulmonary asbestosis, 855
pulmonary, 642
report of 2 cases, *909
Aschheim-Zondek test, modified, for pregnancy, 854
Asthma, death in asthma, with autopsy, 644
transmission of sensitization from mother to child, 494
Astrocytoma of cerebrum showing extensive involvement of opposite cerebral hemisphere, 501
Asymmetry of head and face in infants and in children, 475
Atlas, assimilation of atlas and compression of medulla: clinical significance and pathology of torticollis and localized chronic arthritis deformans of spine, report of case, *333
Autohemagglutination: See Agglutination
Autopsies; postmortem examinations in New York City, recommendations about, 633

B C G: See under Tuberculosis
Bachman precipitin test and human trichinella infections, 296
Bacillus: See Bacteria
Bacteria: See also Diphtheria bacilli; Dysentery, bacilli; Tubercle bacilli; etc.
acidi-lactici, case of endocarditis due to, 672
aerogenes capsulatus, action of congo red on streptococcal and B. welchii hemolysin, 658
aerogenes capsulatus; spores of clostridium welchii, 1030
aertrycke: See Bacteria suipestifer
Brucella abortus, 301
Brucella abortus, cutaneous hypersensitiveness in guinea-pigs infected with Brucella abortus, 131
Brucella abortus from a human fetus, 485
Brucella abortus infection, 1030, 1032
Brucella bacteriophage, 489
Brucella cultures, acidity in, 487
Brucella infections in Tunis and in Malta, 294
colon-aerogenes organisms, 507
Bacteria—Continued
culture media, egg yolk agar medium in tuberculosis, 487
culture medium, Hohn's medium and thick seeding of sediment, 842
do broth culture filtrates contain a bacterial growth-inhibiting substance? 1025
enteritidis; relation of temperature and humidity to course of a B. enteritidis infection in white mice, 651
filtrable forms of, 128
granulosis in relation to trachoma; its pathogenecity for various monkeys and apes, 1033
granulosis, inoculation with, 840
hemolyticus, 1032
lactic acid; meningitis due to bacillus acidilactici in a new-born infant, 1023
metabolism; a summary, *95
of genus hemophilus with regard to X and V growth factors, 650
respiration, some problems of, 1045
Salmonella, agglutination and related phenomena, 657
Salmonella, new serological type of, 658
Salmonella organisms, new lithium selective and enrichment methods for isolation of, 855
sordellii; septic peritonitis in man due to Bacillus sordellii; report of case, *245
suipestifer, changes in blood sugar and blood phosphorus in rabbits following injection of suspensions of, 490
welchii: See Bacteria aerogenes capsulatus
Bacteriology, doctorates in bacteriology and pathology granted by American universities, 1930-1931, 468
Monaco prize for work in bacteriology to Martin R. Veillon, 467
Bacteriophage and enforced dissociation, 486
antigenic properties of, 130
Brucella, 489
specificity of so-called diagnostic bacteriophages, 660
Bacteriotropin, mechanism of action of bacteriotropin; relative degree of phagocytosis by polymorphonuclear leukocytes and by monocytes, 137
Balantidium coli, cultivation, 650
Barker, P. S.: Character of excitation wave in auricular muscle, 134
Bartonella anemia in nonsplenectomized rats, *405
muris anemia, 1026
muris anemia; compensatory phenomena following splenectomy in adult albino rat, 676
muris anemia: pathologic changes during acute anemia, 675
Basedow's Disease: See Goiter, exophthalmic
Beck, C. S.: Pathology of Pick's disease, 138
Becks, H.: Experimental rickets and calcification of dentin, *358
Belding, D. L.: Variations in temperature of healthy and tuberculous birds, 135
Benson, R. L.: Experimental arteritis and arteriosclerosis associated with streptococcal inoculation, *924
Benzene, grave anemia in pregnant women from benzene intoxication, 310
Benzol: See Benzene
Bézi, I.: Assimilation of atlas and compression of medulla; clinical significance and pathology of torticollis and localized chronic arthritis deformans of spine, report of case, *333
Bibliography, departmental bibliography in place of collected reprints, 633
Bier spots, relation of nervous system to a patchy ischaemia (Bier's spots) in animals, 644
Bile ducts, congenital obliteration of, 645
ducts, hamartomas of intrahepatic bile ducts and their relations to cystic liver, 852
peritonitis; chemical changes in blood of dog in experimental bile peritonitis, 482
peritonitis, fat necrosis in, *70
tract, anomaly, 640

Bilirubin, serum and plasma bilirubin: comparative study of 100 cases, 511, 854
Biological Photographic Association, 1014
Biopsy specimens, new technic and instrument for obtaining, 854
Bladder, incidence of cancer of bladder and prostrate in certain occupations, 663
Blair, J. E.: Availability of calcium reserve of spongy trabeculae and compact bone in experimental hyperparathyroidism, 142
Sites of decalcification and bone lesions in experimental hyperparathyroidism, *715
Bloch, B. G.: Experimental infection tuberculosis, 136
Blood: See also Erythrocytes; Leukocytes
action of blood of pregnant women on frog larvae, 831
age of lymphocytes in peripheral blood, 635
alcohol in blood and brain, determination of, 664
bilirubin of plasma and serum, comparative study of 100 cases, 511, 854
changes in blood and renal function after removal of celiac and superior mesenteric ganglions and left adrenal gland, 285
changes in blood sugar and blood phosphorus in rabbits following injection of suspensions of Bact. aertrycke, 490
chemical changes in blood of dog in experimental bile peritonitis, 482
chemistry of blood in thrombo-angiitis obliterans (Buerger), 1021
cholesterol and edema, 647
cholesterol, effect of radiation on blood cholesterol in malignant disease, 307
cholesterol, new method for determination of, 666
comparative toxicity of blood after arteriotomy, asphyxiation and injuries to brain, 281
fibrin, globulin and albumin in blood plasma, method for determination of, 650
fibrinopenia, 119
glutathione in disease, 483
groups, inheritance of, 312
groups, relation between Wassermann reaction and blood groups, 499
hemorrhage and "shock" in traumatized limbs: changes in total, free and bound water of blood and muscle, *869
heredity of agglutinogens M and N of Landsteiner and Levine, with particular reference to determination of paternity, 679
histiocytes in peripheral blood, 477
hydrogen-ion concentration and acid-base equilibrium in normal pregnancy, 482
influence of intravenous injection of urea on exchange of substances between blood and tissues, 831
iodine in blood in exophthalmic goiter, 484
iodine in blood of patients suffering from cancer, 501
iron content of, value of determinations of, 648
lactic acid in blood of newborn, 649
lactic acid in blood in children, 647
nonprotein nitrogen determined by direct nesslerization, 1022
nonprotein sulphur in blood, plasma and serum and glutathione sulphur in blood, 1022
occurrence of serologic factors M and N in Japanese, 1038
oncogenic factor in blood and organs of animals with inoculation tumors, 309
oxalic acid in, 1022
phosphatides and amines of blood in diseases of kidney and their relations to hypertension, 649
plasma; preparation of pure and stable plasma from mammalian blood by centrifugation, 133
plasma protein, red-cell sedimentation and serum lability of blood in tuberculosis, 648
platelet, structure of young blood platelet thrombi, 123
pressure, high; hypertension in cases of congenital polycystic kidney, 116
Blood—Continued
pressure, high; phosphatides and amines of blood in diseases of kidney and their relations to hypertension, 649
pressure, high; pseudohermaphroditismus masculinus externus associated with suprarenal hyperplasia and vascular hypertension, report of case, *543
pressure, micro-injection studies of capillary blood pressure in Raynaud's disease, 284
silicic acid content of blood in tuberculous patients, significance of, 843
solutions, spectrophotometric study of, 139
stains, interpretation of, 311
sugar and lactic acid content of blood changes caused by burns, 280
sugar, radon effects upon blood sugar and its distribution between cells and plasma, 474
transfusion, death after, 667
tubercle bacilliemia in various diseases, 294
tubercle bacilli in blood in laryngeal tuberculosis, 841
typing dried, simple method of, 505
urea in; experimental studies on reticuloendothelial system; influence of india ink on ratio of urea in blood, *180
vessels, proliferation of vascular endothelium, 481
xanthoprotein, lipoids and dextrose in blood serum of persons with cancer, 309
Bodansky, A.: Availability of calcium reserve of spongy trabeculae and compact bone in experimental hyperparathyroidism, 142
Sites of decalcification and of bone lesions in experimental hyperparathyroidism, *715
Bolker, H.: Serum and plasma bilirubin; comparative study of 100 cases, 511
Bollman, J. L.: Act on of epinephrine in depancreatized animals, 141
Bone, availability of calcium reserve of spongy trabeculae and compact bone in experimental hyperparathyroidism, 142
disease, plasma phosphatase in, 126
liposarcoma, primary, 499
marrow; aplasia of marrow and fatal intoxication in dogs produced by roentgen radiation of all bones, 119
marrow, hemendothelioma of spleen and, 501
marrow in agranulocytosis, 142
marrow, panmyelophthisis (atrophy of bone marrow), 124
marrow, reactions of bone marrow in experimentally induced thrombocytosis, *153
ossification; callus formation and calcification, 116
osteogenic sarcoma-like tumor of metacarpal bone, 678
pathologic alterations in bone structure, method of investigating, 666
shadows produced by lead in x-ray pictures of growing skeleton, 287
sites of decalcification and of bone lesions in experimental hyperparathyroidism, *715
syphilis; correlation of roentgenologic picture with anatomic changes in congenital osseous syphilis, 287
tumor; metastasis of "benign" giant-cell tumor of bone, 502
tumors developing after x-ray treatment of bone and joint tuberculosis, 851

Book Reviews:

Anatomie pathologique; M. Letulle, 1048
Antigens in the Light of Recent Investigations; O. Thomsen, 149
Approved Laboratory Technic, Clinical, Pathological, Bacteriological, Serological, Biochemical, Histological; J. A. Kolmer and F. Boerner, 864
Bedside Interpretation of Laboratory Findings; M. G. Wohl, 858
Biologie der Person: Ein Handbuch der allgemeinen und speziellen Konstitutionslehre; T. Brugsch and F. H. Lewy, 329
Chemical Methods in Clinical Medicine; G. A. Harrison, 860

Book Reviews—Continued
Clinical Diagnosis by Laboratory Methods; J. C. Todd and A. H. Sanford, 861
Clinical Interpretation of Blood Examinations; R. A. Kilduffe, 859
Clinical Laboratory Methods; R. L. Haden, 861
Collective review of recent books on clinical pathology, 857
Culture des Tissus en biologie expérimentale; E. C. Craciun, 866
Einführung in die Medizin; H. E. Sigerist, 519
Factor of Infection in the Rheumatic State; A. F. Coburn, 150
Food Poisoning and Food-Borne Infection; E. O. Jordan, 330
Hieronymi Fracastorii de Contagione, et Contagiosis Morbis et eorum Curatione, Libri III; W. C. Wright, 1049
Histogenese ekto-meso-dermaler Mischgeschwülste. Ein Beitrag zur Frage der Organisatorenwirkung (Spemann) beim pathologischen Wachstum; P. Schürmann; H. Pflüger and W. Norrenbrock, 151
Laboratory Medicine; D. Nicholson, 863
Lebensvorgänge im normalen Knorpel und seine Wucherung bei Akromegalie; J. Erdheim, 1047
Lehrbuch der speziellen pathologischen Anatomie für Studierende und Aerzte; E. Kaufmann, 516
Manual of Clinical Laboratory Methods; C. L. Cummer, 861
Manual of Physical and Clinical Diagnosis; O. Seifert and F. Mueller, 857
Morphologie der Missbilungen des Menschen und der Tiere. Ein Hand- und Lehrbuch für Morphologen, Physiologen, Praktische Aerzte und Studierende; G. B. Gruber, 518
Noguchi; G. Eckstein, 517
Oxidation-Reduction Potentials; L. Michaelis, 330
Pathologie und Klinik in Einzeldarstellungen; L. Aschoff; H. Elias; H. Eppinger; C. Sternberg and K. F. Wenckebach, 1047
Physician of the Dance of Death. A Historical Study of the Evolution of the Dance of Death Mythus in Art; A. S. Warthin, 149
Physiopathologie de la thyroïde. Diagnostic et traitement des goitres; L. Dautrebande, 686
Practical Clinical Laboratory Diagnosis Including Interpretation of Laboratory Findings; C. C. Bass and F. M. Johns, 861
Protozoan Parasites of Alimentary Tract; K. M. Lynch, 866
Quantitative Clinical Chemistry; J. P. Peters and D. D. Van Slyke, 859
Recent Advances in Forensic Medicine; S. Smith and J. Glaister, Jr., 329
Regulation of Size as Illustrated in Unicellular Organisms; E. F. Adolph, 328
Renal Lesion in Bright's Disease; T. Addis, and J. Oliver, 685
Resistance to Infectious Diseases: An Exposition of Biological Phenomena Underlying the Occurrence of Infection and Recovery of Animal Body from Infectious Disease, with a Consideration of Principles Underlying Specific Diagnosis and Therapeutic Measures; H. Zinsser, 517
Sexuellen Zwischenstufen; R. Goldschmidt, 148
Textbook of Laboratory Diagnosis with Clinical Applications for Practitioners and Students; E. E. Osgood and H. D. Haskins, 863
Text-Book of Pathology; F. Delafield and T. M. Prudden, 686
Text-Book of Pathology; R. Muir, 685
William Stewart Halsted, Surgeon; W. G. MacCallum, 519
Books Received, 152, 332, 520, 688, 868, 1050
Boor, A. K.: Growth requirements of gonococcus in liquid nonprotein medium, 137
Boxing match, death following, 313
Brain, alcohol in, determination of, 664
and cerebrospinal fluid in experimental cerebral embolism, 117
anemia of, functional disturbances in central nervous system in, 832
astrocytoma of cerebrum showing extensive involvement of opposite cerebral hemisphere, 501
atrophy; pathology of Pick's disease, 138
cadaveric changes of, 504
calcium and phosphorus in brain in different conditions, 482
chondroma; extradural, ventral, 479
dermoid overlying cerebellar vermis with review of literature on intracranial dermoids, 849
diffuse progressive degeneration of gray matter, 120
hemorrhage; apoplexy or suicide, 1043
idiopathic calcification of cerebral vessels, 123
immunologic specificity of antigens of, 146
injuries; comparative toxicity of blood after arteriotomy, asphyxiation and injuries to brain, 281
injury and glioma of, 1040
lesions of brain in influenza, 682
malformation of brain with hydrocephalus and porencephalus, 836
melanoblastosis, primary of, 306
obstruction of aqueduct of Sylvius, 288
posttraumatic tardy hemorrhages of, 665
slicer, *90
specific antigens and antibodies of, 497
traumatic hemorrhage in walls of fourth ventricle, 665
tumor of brain with disturbance in temperature regulation, 116
value of brain lipoids as an index of brain development, 125
Branch, A.: Avian tubercle bacillus infection, with special reference to mammals and to man; its reported association with Hodgkin's disease, *253
Breast, life history of female mammary gland in 2 strains of albino mice, 309
Paget's disease of nipple: review of literature; clinical and microscopic study of 17 breasts by means of whole serial sections, *435
Breedis, C.: Erythroleukosis and anemias of fowl, *1
Breuhaus, H. C.: Homologous liver as a stimulus to hepatic regeneration, *900
Bright's Disease: See Nephritis
Broca's center, relation of, to lefthandedness, 634
Bronchiectasis, isolated giant growth of branch of pulmonary artery associated with congenital bronchiectasis, report of case, *387
Bronchitis, chronic bronchitis with foreign body (elastic fibers) reactions in lungs, *222
Bronchomoniliasis, attempts to produce bronchomoniliasis in monkeys, 651
Bronchus, primary carcinoma of, 850
Brucella: See under Bacteria
Bryant, A. R.: Tularemic leptomeningitis; report of a case, *917
Bucher, C. J.: Asbestosis; report of 2 cases, *909
Burns, changes in sugar and lactic acid content of blood caused by, 280
Butyl alcohol, use of, in preparation of paraffin sections, *599
Byrom, F. B.: Significance of water balance in epilepsy, 139

Cadavers, dermographic examinations of dead bodies, 311
Calcification, of subcutaneous tissue ("calcinosis universalis") in a child, 634
Calcium, absorption from gallbladder, 830
and phosphorus in brain in different conditions, 482
effect of irradiated ergosterol and increased calcium on growth of tumor tissue, 308
Callus formation; ossification; and calcification, 116

Cancer: See also Sarcoma; Tumor; etc.; and under names of organs and regions
adenoid cystic carcinoma with generalized metastases in 3 cases of basal cell type, 1039
alteration of malignancy in metastatic growths after removal or irradiation of primary growths, 663
biochemical studies of, 846
carbohydrate tolerance and alkalosis in malignant disease, 308
Citelli-Piazza reaction in malignant tumors, 502
carcinoid tumors of small intestine, 1040
complement-fixation experiments with alcoholic cancer extracts and with other synthetic antigens, 498
complement fixation with alcoholic carcinoma extracts, 853
development from polypi of colon, 138
effect of certain internal secretions on growth of, 850
effect of radiation on blood cholesterol in malignant disease, 307
effect of eu- and pseudo-globulin fractions of anti-cancer sera on tissue cultures, 502
etiology, tissue transplantation and parasitism, *983
in Hamburg, 1041
influence of aqueous extract of suprarenal cortex on growth of, 847
influence of extracts of suprarenal cortex on growth of carcinoma, sarcoma and melanoma, 847
influence of suprarenalin (epinephrin) on growth of carcinoma and sarcoma in animals, 307
iodine content of blood of patients with, 501
lipoid metabolism in, 853
mice as indicators of carcinogenic noxae, 310
occurrence of malignant growth in persons with radioactive deposits, 511
pellagra secondary to benign and carcinomatous lesions and to dysfunction of gastro-intestinal tract, 281
roentgen carcinoma and sarcoma, 849
serological diagnosis of, 307
squamous cell carcinoma in a dermoid cyst of ovary, 144
statistics in New York State for four-year period 1925-1928, 308
tar cancer in mice, effects of dyes on, 503
tar, experimental tar tumors in white rat, 850
xanthoprotein, lipoids and dextrose in blood serum of persons with, 309
Cannon, P. R.: Immunity of tissues; conditions influencing fixation and destruction of bacteria in tissues, 138
Carbohydrate tolerance and alkalosis in malignant disease, 308
Carbon Monoxide, detection of carbon monoxide in exhumed bodies, 506
use of illuminating gas in preparation of museum specimens, 133
Cardiovascular renal disease; report of a case of 3000 years ago, *92
Carotid body, tumors of, 661
Carotinoid pigments in birds, 323
Cartilage, degeneration of articular cartilage in bovine joints, 1018
effects of graded degrees of heat on cartilage and on thyroid gland, *590
heterotransplantation of, *590
homoiotransplantation of, *590
in salivary gland, *31
Cells, division: mitogenetic radiation, 472
induced development and histogenesis of plasma cells, 1019
Cellulitus, sporadic, and abscess due to streptococcus epidemicus, 1031
Cerebrospinal Fluid and brain in experimental cerebral embolism, 117
bloody spinal fluid following an osteopathic treatment, 1042
precipitation with, 127
Cheatle, G. L.: Paget's disease of nipple; review of literature; clinical and microscopic study of 17 breasts by means of whole serial sections, *435
Chickenpox-herpes zoster virus, 293
variola-vaccinia flocculation, 659
Cholelithiasis: See Gallbladder calculi
Cholesterol and edema, 647
in blood: See under Blood
pathogenesis of irradiated cholesterol poisoning, 639
Chondroma, extradural ventral, 479
Chondrosarcoma with intravascular growth and tumor emboli to lungs, 500
Chordoma, sacral, 661
Chromoblastomycosis, pulmonary "pseudo-actinomycosis" (chromoblastomycosis?) in a capybara due to a hyphomycete producing ray fungi in tissue, 326
Cinchopen poisoning, specific character of toxic cirrhosis in, 640
Citelli-Piazza reaction in malignant tumors, 502
Clawson, B. J.: Nonsyphilitic aortic valve deformity, *889
Clostridium Tetani: See under Tetanus
Welchii: See under Bacteria aerogenes capsulatus
Clute, H. M.: Cancer of thyroid, 316
Colds, bacteriological study of "colds" on an isolated tropical island, 292
common, etiology of, 291
Coleman, E. H.: Asbestosis; report of 2 cases, *909
Collodion as an instrument of suicide, 1042
membranes for bacteriologic use, 855
Colloid bodies in living body and in tissue cultures, phagocytosis of, 639
Colon, development of carcinoma from polypi of, 138
Columbia University, departmental bibliography in place of collected reprints, 633
Congo red, action of congo red on streptococci and B. welchii hemolysin, 658
Copper and iron in immunity, 655
in human and bovine milk, 1022
Cornea, syndrome characterized by congenital clouding of cornea and by other anomalies, 470
Corson-White, E. P.: Carotinoid pigments in birds, 323
Cranium, atypical gunshot wounds of skull, 313
injuries, lucid interval before death after, 310
roentgenograms in study of intracranial hemorrhage in new-born, 644
tumor of posterior cranial fossa causing visual hallucinations, 289
xanthomatosis with cranial defects (Schüller's disease), 832
Custer, R. P.: Nature of erythrocytic damage produced by acetylphenylhydrazine in vivo and in vitro, 326
Cutler, M.: Paget's disease of nipple; review of literature; clinical and microscopic study of 17 breasts by means of whole serial sections, *435
Cyanosis, vascular mechanism in acrocyanosis, 284
Cystadenomas, malignant cystadenomas of liver, 853
Cysts, congenital air cyst of lung, 476
intrathoracic, 480

Damon, L. A.: Histogenesis of amyloid bodies in central nervous system, *229
Dehydration, influence of dehydration on anaphylaxis in guinea pigs, 1035
Delaney, P. A.: Mixed tumor of scalp, 145
Squamous cell carcinoma in dermoid cyst of ovary, 144
Dengue, location of dengue virus in body of mosquitoes, 651
Dentin, experimental rickets and calcification of, *358
Dermatographic examination of dead bodies, 311
Dermoid overlying cerebellar vermis with review of literature on intracranial dermoids, 849

Dermoid—Continued
squamous cell carcinoma in dermoid cyst of ovary, 144
Diabetes insipidus, studies on, 280
sugar content of skin and muscle in diabetic and nondiabetic persons, 126
Diaphragm, anomaly, rare, 834
Diarrhea, etiology of infectious diarrhea (winter scours) in cattle, 1025
Dickar, L.: Endocarditis due to bacterium acidi-lactici, 672
Diet, effect of diet in epidemic infections in mice, 490
Diphtheria, antibodies in urine after injection of antitoxic globulin, 298
antitoxin, absorption of, 303
antitoxin in human saliva, 494
antitoxin in human sweat, 1036
fractions of different antitoxic quality from same serum, 658
intracutaneous method of testing diphtheria toxin and antitoxin, 303
loss of diphtheria antitoxin in human urine, 493
mechanism of toxin-antitoxin reactions, 1036
neutralization or destruction of diphtheria toxin by tissue, 654
precipitins in diphtheria immune serum, 2 kinds, 498
specific (immunologic) properties of purified toxins, 1038
toxin, 1032
toxin, acid precipitation, 494
toxin, infusion and infusion-free, 494
toxin, purification and concentration of, 305
toxin, speed of flocculation of, 494
Diphyllobothrium latum, native infestation with, 839
Disease, genetics and pathology, 637
Distomiasis, hepatic, specific Wassermann reaction in, 496
Doctorates in bacteriology and pathology granted by American universities, 1930-1931, 468
Donaldson, L.: Cellular reaction to infection with tubercle bacilli; experiments on testes of normal and immunized guinea-pigs, 135
Early cellular reaction to tubercle bacilli; a comparison of this reaction in normal and tuberculous guinea-pigs and guinea-pigs immunized with dead bacilli, *956
Downs, W. G., Jr.: Rôle of anterior lobe of pituitary gland in growth; with reference to teeth and maxillae, *37
DuBois, F. S.: Chronic bronchitis with foreign body (elastic fibers) reactions in lungs, *222
Ductus arteriosus, aberrant, congenital tracheoesophageal fistula caused by, 508
arteriosus, patent, with primary bacterial pulmonary endarteritis, 476
Duodenum, carcinoma, primary, 848
does carcinoma of duodenum ever arise from duodenal ulcers, 848
obstruction caused by congenital bands, 476
Duval, C. W.: Experimental scarlatinal nephritis in dog, *572
Dyes: See also Stains and Staining
effects of dyes on tar cancer in mice, 503
Dysentery, antigens of Shiga bacillus, 844
heterogenetic antigen of Shiga bacillus, 844
specific precipitating polysaccharide from B. dysenteriae (Shiga), 657
toxin of Shiga bacillus, 843
Dystrophy, muscular nerve endings in, 635
muscular, nutritional, 140, 635

Ear, direct communications between arteries and veins in rabbits, 285
Echinococcosis, pathologic anatomy of multilocular echinococcus cyst in liver, 1020
Ectodermoses: See Skin diseases
Edema and cholesterol, 647
erythroblastosis with jaundice and edema in newly born, 641
experimental nephrotic, 1015
Eggers, H. E.: Cartilage in a salivary gland, *31
Eggers, H. E.—Continued
Etiology of cancer; tissue transplantation and parasitism, *983
Electricity, accidental injuries by electric currents, 312
medicolegal importance of certain new electropathologic findings, 1044
Electrolytes, distribution of electrolytes in serum during immunization, 495
effect on pulmonary epithelium of intrapleural injections of, 471
Embolism, cerebral, brain and cerebrospinal fluid in, 117
chondrosarcoma with intravascular growth and tumor emboli to lungs, 500
malignant endocarditis (pneumococcal) with early calcification and calcareous renal emboli, 833
Embryos, repair in vitro of embryonic skeletal rudiments after experimental injury, 644
Encephalitis: See also Encephalomyelitis
and encephalomyelitis in measles, 122
lethargic: Glasgow epidemic of 1923, 653
medicolegal significance of, 311
periaxialis diffusa (Schilder's disease), 643
relation of streptococci to filtrable virus of epizootic encephalitis of fox, 488
Encephalomalacia, histopathology of nutritional encephalomalacia of chicks, 1019
nutritional, in chicks, 134
Encephalomyelitis and encephalitis in measles, 122
disseminated, 121
postvaccinal, etiology of, *601
Endarteritis, patent ductus arteriosus with primary bacterial pulmonary endarteritis, 476
Endocarditis, due to bacterium acidi-lactici, 672
endocarditis process in childhood, *49
malignant endocarditis (pneumococcal), with early calcification and calcareous renal emboli, 833
Endocrine Glands, effect of certain internal secretions on growth of cancer, 850
effect of internal secretions upon division energy of paramecia, 308
Endometrioma, origin of: experimental study with case reports, 480
Endometriosis, experimental production of, 833
Endothelioma, hemendothelioma of spleen and bone marrow, 501
Endothelium, vascular, proliferation of, 481
Enteritis: See Intestine, disease
Epidermis: See Skin
Epilepsy, significance of water balance in, 139
Epinephrin: See Suprarenal preparations
Epithelioid cells, 634
Ergosterol, effect of irradiated ergosterol and increased calcium on growth of tumor tissue, 308
experimental ergosterol poisoning, 1018
hypervitaminosis D and arteriosclerosis, *941
Erythema nodosum, tubercle bacilli in children with, 1023
Erythroblastosis, is familial jaundice of newborn infants erythroblastosis, 120
with jaundice and edema in newly born, 641
Erythrocytes, catalase action and glutathione content of erythrocytes in anemias, 649
effect of injection of trypan blue on rate of sedimentation of erythrocytes in inflammation, *429
effect of testicle extract on red cells, 635
Erythroleukosis and anemias of fowl, *1
Esophagus, atresia, congenital, with tracheoesophageal fistula, report of 8 cases, *756
myoma of esophagus with associated diverticula; report of 2 instances, *77
sarcoma of, 1040

Face, asymmetry of head and face in infants and in children, 475
Falls, experimental studies of high plunge into water, 312
Fat metabolism, experimental studies in, 484
necrosis in bile peritonitis: experimental study, *70
Feces, asbestosis bodies in feces in pulmonary asbestosis, 855

Feces—Continued
flora of adults, 1032
lead in feces and urine, 649
Fennel, E. A.: Familial congenital atresia of intestine, 147
Ferraro, A.: Histogenesis of amyloid bodies in central nervous system, *229
Ferrosilicon, poisoning from, 504
Fetus, Brucella abortus from a human fetus, 485
Fibroblastoma, meningeal fibroblastoma (dural endothelioma, meningioma, arachnoid fibroblastoma), 661
Fibrinopenia: See Blood fibrinogen
Filarial periodicity, 129
Fishback, F. C.: Experimental pathology of liver, *787
Fistula, congenital absence of 1 kidney with urethrorectal fistula, urethral stone and concomitant measles, 315
tracheo-esophageal, congenital, caused by aberrant ductus arteriosus, 508
tracheo-esophageal, with congenital atresia of esophagus, report of 8 cases, *756
Flocculation-reaction time in course of immunization and changes of proteins, 495
Fluids, magnetic susceptibility of body fluids, 483
Foreign Body, chronic bronchitis with foreign body (elastic fibers) reactions in lungs, *222
tooth in pleural cavity, 288
Fried, B. M.: Infection of rabbits with tubercle bacillus by way of trachea; studies on defensive and metabolic apparatus of lungs, *689
Frog larvae, action of blood of pregnant women on, 831
Fungi, hypersensitiveness to soluble specific substances from yeast-like fungi, 470
Furth, J.: Erythroleukosis and anemias of fowl, *1

Galactose in tests of function of liver, 506
Gallbladder, absorption of calcium from, 830
calculi, heavy metals in, 128
function, 1016
two in 1 person, 835
Ganglions, changes in blood and renal function after removal of celiac and superior mesenteric ganglions and left adrenal gland, 285
Gangrene, sclerosis of small mesenteric vessels with ulceration and gangrene of enteric tract, 833
Gastro-Intestinal Tract, pellagra secondary to benign and carcinomatous lesions and to dysfunction of, 281
sclerosis of small mesenteric vessels with ulceration and gangrene of enteric tract, 833
Gaucher's Disease: See Anemia, splenic
Gay, J. G.: Experimental pathology of liver, *787
Genetics and pathology, 637
Genitals, rape and deformity of male sexual organs, 314
Glia cells: See Neuroglia
Gliomas, calcification in, 849
injury and glioma of brain, 1040
Glomerulonephritis: See under Nephritis
Glucose, influence of x-ray lesions of intestinal mucosa on absorption of glucose and other sugars, 471
Glycerol, effect on growth of tubercle bacillus of modification of amount of glycerol in tissues, 1024
Goettsch, M.: Nutritional encephalomalacia in chicks, 134
Nutritional muscular dystrophy in guinea-pigs and rabbits, 140
Goiter, exophthalmic, iodine in blood in, 484
Goldzieher, M. A.: Experimental studies on reticulo-endothelial system; influence of india ink on ratio of urea in blood, *180
Reticulosis, *773
Gonococcus, growth requirements of gonococcus in liquid nonprotein medium, 137
Goodman, R.: Foreign body in orbit, 676
Graham, J. E.: Periarteritis nodosa complicated by fatal intrapericardial hemorrhage; report of case, *521
Grant for study of renal function, 467
Gray, S. H.: Septic peritonitis in man due to bacillus sordellii; report of case, *245
Green, G. F.; Experimental pathology of liver, *787
Gruhzit, O. M.: Relation of sulphur to experimental anemia, 140
Gunn, F. D.: Reactions of bone marrow in experimentally induced thrombocytosis, *153
Guy, C. C.: Congenital diaphragmatic hernia associated with an accessory lung, 146
Pseudohermaphroditismus masculinus externus associated with suprarenal hyperplasia and vascular hypertension, report of case, *543

Hall, I. C.: Septic peritonitis in man due to bacillus sordellii; report of case, *245
Hamartomas of intrahepatic bile ducts and their relation to cystic liver, 852
Hay fever, transmission of sensitization from mother to child, 494
Haythorn, S. R.: Cytologic reactions in experimental monilial abscesses, 135
Head, asymmetry of head and face in infants and in children, 475
flat head and nonrachitic soft chest, a new syndrome, 475
injuries by blunt force, 313, 505
Healey, C. E.: Pseudohermaphroditismus masculinus externus associated with suprarenal hyperplasia and vascular hypertension, report of case, *543
Heart, acquired rheumatic pulmonic stenosis and insufficiency, 476
character of excitation wave in auricular muscle, 134
functionally two-chambered, 475
increase in heart volume in mitral stenosis, 123
injections into, complications following, 1044
morbid anatomy in hyperthyroidism, 478
myxoma of, 647
neoplasm of heart valve, primary, 500
nonsyphilitic aortic valve deformity, *889
persistent ostium atroventriculare commune with septal defects in a Mongolian idiot, 640
rhabdomyoma, congenital, 500
Heat, effects of graded degrees of heat on cartilage and on thyroid gland, *590
skin reactions produced by alterations of heat and x-ray at various time intervals, 118
Hemachromatosis, brown pigment of, 483
Hemagglutination: See Agglutination
Hemangioblastomas, cerebellar, with incidental changes of spinal cord, 122
Hemangio-endothelioma, intravascular hemangio-endothelioma of ovary, 661
Hemangioma, malignant, of lung with multiple visceral foci, report of case, *562
Hematopoietic System, combined effects of colloidal silver and highly filtered roentgen radiation upon hematopoietic system in dogs, 119
effects of intravenous injection of colloidal silver upon hematopoietic system in dogs, 118
Hemendothelioma: See Endothelioma
Hemoglobin, precipitins, specificness of, 656
Hemorrhage and "shock" in traumatized limbs: changes in total, free and bound water of blood and muscle, *869
effects of hemorrhage on vascular nervous mechanism, 636
mechanism of sudden death following acute circulatory disturbances, 1043
Hemorrhagic diatheses, 286, 837
Hepatitis: See Liver
Hermaphroditism, pseudohermaphroditismus masculinus externus associated with suprarenal hyperplasia and vascular hypertension, report of case, *543
Hernia, diaphragmatic, congenital, associated with accessory lung, 146
Herpes zoster and chickenpox-virus, 293
Hewitt, W. A.: Use of butyl alcohol in preparation of paraffin sections, *599

Higgins, G. M.: Experimental pathology of liver; restoration of liver of white rat following partial surgical removal, *186
Hirsch, E. F.: Tularemic leptomeningitis; report of a case, *917
Histiocytes, appearance in peripheral blood, 477
Histology, frozen unfixed tissue sections for histologic study, 666
Hodgkin's Disease: See Lymphogranuloma
Hohn's medium and thick seeding of sediment, 842
Hooker, H. F.: Gaucher's disease, 146
Hornick, O. S.: Reticulosis, *773
Hydatid Cyst: See Echinococcosis
Hydrocephalus, malformation of brain with hydrocephalus and porencephalus, 836
Hydrogen ion concentration and acid-base equilibrium in normal pregnancy, 482
Hydrophobia: See Rabies
Hyperinsulinism from B-cell adenoma of pancreas, 282
Hypersensitiveness: See Anaphylaxis and Allergy
Hypertension: See Blood pressure, high
Hyperthyroidism: See Thyroid

Icterus: See Jaundice
Idiot, persistent ostium atrioventriculare commune with septal defects in a Mongolian idiot, 640
Illuminating Gas: See Carbon monoxide
Immunity: See also Anaphylaxis and Allergy
  an aspect of inflammation in relation to, *802
  copper and iron in, 655
  distribution of electrolytes in serum during immunization, 495
  flocculation-reaction time in course of immunization and changes of proteins, 495
  influence of hypersensitiveness to horse serum on duration of local passive antitoxic immunity in man, 300
  of tissues: conditions influencing fixation and destruction of bacteria in tissues, 138
  quantitative aspects of immunity reactions, 656
  rôle of nervous system and of conditional reflexes in, 496
  simultaneous multiple immunization, 656
  transmission to third generation of antitoxin, 300
India ink, experimental studies on reticuloendothelial system; influence of india ink on ratio of urea in blood, *180
Individuality differentials in strains of inbred rats, *203
Industries, chronic industrial radium poisoning in Joachimsthal, 665
  incidence of cancer of bladder and prostate in certain occupations, 663
  occupational neoplastic diseases, 847
Infant, new-born, erythroblastosis with jaundice and edema in, 641
  new-born, is familial jaundice of new-born infants erythroblastosis? 120
  new-born, lactic acid in blood of, 649
  new-born, roentgenograms in study of intracranial hemorrhage in, 644
Infections, effect of diet in epidemic infections in mice, 490
  focal, and menstruation, 141
Inflammation, an aspect of inflammation in relation to immunity, *802
  effect of injection of trypan blue on rate of sedimentation of erythrocytes in, *429
  fixation of bacteria and of particulate matter at site of, 283
  generalized changes in, 638
Influenza, bacillus influenzae, variation and type specificity in species hemophilus influenzae, 291
  brain, lesions of, 682
Instrument for obtaining specimen for biopsy, 854
Insulin, occurrence of insulin in urine, 1022
  poisoning as a cause of an automobile accident, 314
Internal Secretions: See Endocrine Glands
Intestine, adenoma in swine, 500
  atresia of, familial, congenital, 147
Intestine—Continued
  bacterial flora of intestinal segments, 488
  carcinoid tumors of, 1040
  diseases, vibrionic enteritis in calves, 1026
  flora; lysozyme in development of intestinal flora of new-born infant, 487
  flora of adults, 1032
  influence of x-ray lesions of intestinal mucosa on absorption of glucose and other sugars, 471
  obstruction from endometrial proliferation in intestinal wall, 481
  vibrios (vibrio jejuni, n. sp.) associated with intestinal disorders of cows and calves, 1026
Iodine content of blood of patients suffering from cancer, 501
  in blood in exophthalmic goiter, 484
Iron, accumulation of iron in tuberculous areas, 1027
  and copper in immunity, 655
  value of determinations of iron content of whole blood, 648
Isaacs, R.: Bone marrow in agranulocytosis, 142
Ischemia, relation of nervous system to a patchy ischemia (Bier's spots) in animals, 644
Isohemagglutination: See Agglutination

Jacobi, M.: Congenital tracheo-esophageal fistula caused by an aberrant ductus arteriosus, 508
  serum and plasma bilirubin; comparative study of 100 cases, 511
Jaffe, H. L.: Availability of calcium reserve of spongy trabeculae and compact bone in experimental hyperparathyroidism, 142
  Osteogenic sarcoma-like tumor of metacarpal bone, 678
  Sites of decalcification and of bone lesions in experimental hyperparathyroidism, *715
Jaundice and erythroblastosis and edema in newly born, 641
  experimental obstructive, 832
  is familial jaundice of new-born infants erythroblastosis? 120
  Leptospira icterohemorrhagiae in wild rats, 839
  obstructive, blood-pigment in, 127
  of new-born, causes of icterus neonatorum, 636
Jaws, rôle of anterior lobe of pituitary gland in growth with reference to teeth and maxillae, *37
Joints, degeneration of articular cartilage in bovine joints, 1018
Jungeblut, C. W.: Effect of filtration and of various constituents of tissue on virus of poliomyelitis, 137
Juxta-articular nodules, subcutaneous, 478

Kendall, A. I.: Bacterial metabolism; a summary, *95
Kennedy, R. P.: A spectrophotometric study of blood solutions, 139
Kidney, absence of 1 kidney, congenital, with urethrorectal fistula, urethral stone and concomitant measles, 315
  calcification, 833
  changes in blood and renal function after removal of celiac and superior mesenteric ganglions and left adrenal gland, 285
  denervation: effect of snake venom; effect of repeated chillings, 142
  disease, cardiovascular renal disease; report of a case of 3,000 years ago, *92
  experimental nephrotic edema, 1015
  function, grant for study of, 467
  innervation of renal parenchyma, 280
  malignant endocarditis (pneumococcal) with early calcification and calcareous renal emboli, 833
  normal renal glomerulus of man; histologic considerations, *413
  number of open glomeruli in acute mercuric chloride nephrosis, 123

Kidney—Continued
phosphatides and amines of blood in diseases of kidney and their relations to hypertension, 649
polycystic, hypertension in cases of, 116
King, H. D.: Individuality differentials in strains of inbred rats, *203
Kintner, A. R.: Anomalous origin and course of left coronary artery, *586
Klinck, G. H., Jr.: Effect of injection of trypan blue on rate of sedimentation of erythrocytes in inflammation, *429
Klingelhofer, R.: Erythroleukosis and anemias of fowl, *1
Kraft, E.: Gaucher's disease, 146
Krumbhaar, E. B.: Hodgkin's disease of bone marrow and spleen without apparent involvement of lymph nodes, 322

Lactic Acid: See Acid, lactic
Lang, F. J.: Generalized osteitis fibrosa and its relation to osteomalacia and rickets, 1045
Larynx, tubercle bacilli in blood in laryngeal tuberculosis, 841
Lead, behavior in animal organism, 830
detection of small quantities in tissues, 307
in feces and urine, 649
shadows produced by lead in x-ray pictures of growing skeleton, 287
Lederer, M.: Heredity of agglutinogens M and N of Landsteiner and Levine, with particular reference to determination of paternity, 679
Psittacosis; postmortem examination of case, including studies on spinal cord, 319
Two unusual cases of interstitial myocarditis, 668
Lefthandedness, relation of Broca's center to, 634
Leprosy, morbid anatomy and pathogenesis of, 479
Leptomeninges, primary melanoblastosis of leptomeninges and brain, 306
tularemic: report of a case, *917
Leptospira Icterohemorrhagiae: See under Jaundice
Leukemia, lymphatic, smear preparations in, 666
lymphoblastic, in infant, fatal, 474
mouse, 662
transmissible lymphoid leucemia of mice, 284
Leukocytes, experimental leukosis, in fowl, 639
mechanism of action of bacteriotropin: relative degree of phagocytosis by polymorphonuclear leukocytes and by monocytes, 137
Leukosis: See under Leukocytes
Levinson, A.: Effects of intrathecal administration of mercurochrome-220 soluble and of metaphen, *729
Lewis, J. H.: Immunologic specificity of antigens of brain, 146
Lipoid cell hyperplasia of spleen, 1020
deposits in aortas of infants, 645
metabolism in cancer, 853
metabolism, studies in, 483
value of brain lipoids as an index of brain development, 125
xanthoprotein, lipoids and dextrose in blood serum of persons with cancer, 309
Lipoma, retrorectal, 646
Liposarcoma of bone, primary, 499
Liver, acute yellow atrophy of liver from poisoning by morel (Helvella esculenta), 504
calcification; intrahepatic lithiasis, 640
comparison of vital staining of parenchyma cells of liver with acid and basic dyes, 483
cystadenomas of, malignant, 853
cystic, harmartomas of intrahepatic bile ducts and their relations to, 852
detoxicating function of liver with special reference to strychnine, 281
distomiasis, specific Wassermann reaction in, 496
echinococcus cyst in, pathologic anatomy of, 1020

Liver—Continued
enlargement, splenomegaly and hepatic enlargement in hereditary hemorrhagic telangiectasia, 116
experimental pathology, *787
experimental pathology, restoration of liver of white rat following partial surgical removal, *186
function, galactose in tests of, 506
granulomatous abscess of liver of pyogenic origin, 640
homologous liver as a stimulus to hepatic regeneration, *900
necrosis from indirect injury, 664
rôle of diet in protecting against liver injury by arsphenamine, 830
rôle of liver in formation of lymph, 281
streptococcus hepatitis, 641
therapy with liver: See under Anemia
Loeb, L.: Individuality differentials in strains of inbred rats, *203
Long, A. R.: Cardiovascular renal disease; report of case of 3000 years ago, *92
Long, E. R.: Cellular reaction to infection with tubercle bacilli; experiments on testes of normal and immunized guinea-pigs, 135
Early cellular reaction to tubercle bacilli; a comparison of this reaction in normal and tuberculous guinea-pigs and guinea-pigs immunized with dead bacilli, *956
Lucké, B.: Mechanism of action of bacteriotropin; relative degree of phagocytosis by polymorphonuclear leukocytes and by monocytes, 137
Lung abscesses, multiple, simulating tuberculosis, 485
accessory; congenital diaphragmatic hernia associated with accessory lung, 146
asbestosis bodies in feces in pulmonary asbestosis, 855
changes after removal of cervical part of sympathetic nerve, 285
chondrosarcoma with intravascular growth and tumor emboli to, 500
chronic bronchitis with foreign body (elastic fibers) reactions in lungs, *222
congenital air cyst of, 476
effect on pulmonary epithelium of intrapleural injections of electrolytes, 471
fatty changes in pulmonary veins, 834
immunity in lungs of rabbits following immunization with pneumococci, 302
infection of rabbits with tubercle bacillus by way of trachea; studies on defensive and metabolic apparatus of lungs, *689
malignant disease of mediastinal glands, lungs and pleurae, morbid anatomy of, 644
malignant hemangioma of lung with multiple visceral foci, report of case, *562
multiple tuberous osteomas of, 836
"pseudoactinomycosis" (chromoblastomycosis?) in a capybara due to a hyphomycete producing ray fungi in tissue, 326
putrefying, gas analysis of, 505
significance of lungs for transportation of material within body, 831
Lymph, rôle of liver in formation of, 281
Lymph Nodes, bronchial, latent and occult tuberculosis of bronchial glands, 489
inoculation of tuberculous material into lymphatic ganglions, 133
tuberculosis in mesenteric lymph nodes of children, 484
Lymphatism, analysis of data collected by status lymphaticus investigation committee, 285
significance of thymic and thymicolymphatic status in legal medicine, 310
status thymicolymphaticus in legal medicine, 1042
Lymphocytes, age of lymphocytes in peripheral blood, 635
Lymphocytosis, induction of lymphocytosis and lymphatic hyperplasia by means of protein, 282

Lymphogranuloma, avian tubercle bacillus infection with special reference to mammals and man; its reported association with Hodgkin's disease, *253
case of Hodgkin's disease of unusual duration and extension, 323
Hodgkin's diseases of bone marrow and spleen without apparent involvement of lymph nodes, 322
Lymphoid hyperplasia, malignant lymphoid hyperplasia in mice, 307
Lymphosarcoma of neck, 663
Lysozyme in development of intestinal flora of new-born infant, 487

McCutcheon, M.: Mechanism of action of bacteriotropin; relative degree of phagocytes by polymorphonuclear leukocytes and by monocytes, 137
McJunkin, F. A.: Effect of homologous macerated skin on regeneration of epidermis, *794
Homologous liver as a stimulus to hepatic regeneration, *900
Macleod, A. G.: Character of excitation wave in auricular muscle, 134
Magnesium, action of magnesium on adenocarcinoma of rat, 309
Magnetic susceptibility of body fluids, 483
Malaria, pernicious, 650
Malta Fever: See Undulant fever
Mann, F. C.: Experimental pathology of liver, *787
Marmorston-Gottesman, J.: Bartonella muris anemia; compensatory phenomena following splenectomy in adult albino rat, 676
Bartonella muris anemia; pathologic changes during acute anemia, 675
Martland, H. S.: Occurrence of malignant growth in persons with radioactive deposits, 511
Mastoiditis and otitis media due to streptococcus epidemicus, 1031
Matsui, T.: Effect of homologous macerated skin on regeneration of epidermis, *794
Mayer, L.: Osteogenic sarcoma-like tumor of metacarpal bone, 678
Measles, case of congenital absence of 1 kidney, with urethrorectal fistula, urethral stone and concomitant measles, 315
encephalitis and encephalomyelitis in, 122
serum prophylaxis in, 305
Mediastinum, morbid anatomy of malignant disease of mediastinal glands, lungs and pleurae, 644
teratomas of, 2 cases, 480
Medium: See Bacteria culture media
Medulla oblongata; assimilation of atlas and compression of medulla, clinical significance and pathology of torticollis and localized chronic arthritis deformans of spine; report of a case, *333
Melanoblastosis, primary melanoblastosis of leptomeninges and brain, 306
Melanoma, influence of aqueous extract of suprarenal cortex on growth of, 847
influence of extracts of suprarenal cortex on growth of, 847
Meninges, meningeal fibroblastoma (dural endothelioma, meningioma, arachnoid fibroblastoma), 661
tumors, origin and nature of, 846
Meningitis due to bacillus acidi-lactici in new-born infant, 1023
Meningococcus antiserum; neutralization of skin reaction of antimeningococcus serums, 301
filtrate, skin reaction to agar after injection of, 131
Menkin, V.: An aspect of inflammation in relation to immunity, *802
Menstruation and focal reactions, 141
Mercuric chloride, number of open glomeruli in acute mercuric chloride nephrosis, 123
Mercurochrome, effects of intrathecal administration of mercurochrome-220 soluble and of metaphen, *729
Mesentery, tuberculosis in mesenteric lymph nodes, 484
Metabolism, basal, effects of posterior pituitary extracts on, 281
basal, of Maya Indians in Yucatan, 281
rôle of spleen in, 639
Metacarpus, osteogenic sarcoma-like tumor of metacarpal bone, 678
Metaphen, effects of intrathecal administration, *729
Micrococcus lysodeikticus, action of lysozyme on, 845
Microglia: See Neuroglia
Milk antibodies in serum of nurslings, 496
bacteriologic examination of, 489
copper in human and bovine milk, 1022
rôle of alcohol-soluble constituents of milk in its serologic reactions, 845
Miller, C. P., Jr.: Growth requirements of gonococcus in liquid nonprotein medium, 137
Miles, G.: Renal denervation; effect of snake venom; effect of repeated chillings, 142
Milliken, M.: Menstruation and focal reactions, 141
Mongolism in one of twin, 116
Monilia, cytologic reactions in experimental monilial abscesses, 136
serological classification of, 657
Monkeys born in captivity, 285
Monocytes in tuberculous animals, 1025
Morel, acute yellow atrophy of liver from poisoning by, 504
Mudd, S.: Mechanism of action of bacteriotropin; relative degree of phagocytosis by polymorphonuclear leukocytes and by monocytes, 137
Müller, E. F.: Renal denervation; effect of snake venom; effect of repeated chillings, 142
Mumps: See Parotitis
Muscle, calcium content of striated muscle of rachitic animals, 126
hemorrhage and "shock" in traumatized limbs: changes in total, free and bound water of blood and, *869
sugar content of skin and muscle in diabetic and non-diabetic persons, 126
Museum specimens, use of illuminating gas in preparation of, 133
Myatonia, amyotonia with histologic observations, 287
Myeloma, diffuse myeloma with amyloid masses, 646
Myocarditis, acute isolated, 477
interstitial, 2 unusual cases of, 668
Myocardium in yellow fever, 636
Myoma of esophagus with associated diverticula: report of 2 instances, *77
Myxoma of heart, 647

National Research Council, medical fellowship board, meets in March, 1014
Neck, lymphosarcoma of, 663
Neckermann, E. F.: Immunity of tissues; conditions influencing fixation and destruction of bacteria in tissues, 138
Nephritis, experimental, in frog, 470
experimental scarlatinal nephritis in dog, *572
glomerular; experimental acute glomerulitis, 282
mitochondrial changes in oxalate and uranium nephritis, 644
Nephroma, embryonal, in chicken, 846
Nephrosclerosis, 1015
Nerve, cervical sympathetic, changes in lung after removal of cervical part of sympathetic, 285
Nervous System, functional disturbances in central nervous system in cerebral anemia, 832
histogenesis of amyloid bodies in central nervous system; rôle of microglia in formation of amyloid body, *229
rôle of nervous system and of conditional reflexes in immunity, 496
Neurofibromatosis, multiple, 1039
Neuroglia, experimental studies of glia cells, 481
rôle of microglia in formation of amyloid body, *229
Neuromyo-arterial glomus (Masson), tumors of, 852

Neurosyphilis: See Syphilis
New-Born: See under Infant
Nitrogen, determination of nonprotein nitrogen by direct nesslerization, 1022

Obituaries:
Agramonte, A., 467
Kitasato, S., 115
Warthin, A. S., 276
Occupations: See Industries
Olcott, C. T.: Carcinoma of suprarenal cortex with unusual metastases, 319
Oligodendroglia: See Neuroglia
Oligodendroglioma, 1039
Omentum, absorption of particulate matter by great omentum, 283
Orbit, foreign body in, 676
Osteitis, generalized osteitis fibrosa and its relation to osteomalacia and rickets, 1045
fibrosa, experimental chronic hyperparathyroidism leading to, 283
Osteomalacia, carcinomatous, histologic changes in, 853
generalized osteitis fibrosa and its relation to osteomalacia and rickets, 1045
Osteomas, multiple tuberous osteomas of lung, 836
Osteopathy, bloody spinal fluid following an osteopathic treatment, 1042
Otitis media and mastoiditis due to streptococcus epidemicus, 1031
Ovary, intravascular hemangio endothelioma of, 661
primary malignant adamantinoma of, 1041
squamous cell carcinoma in a dermoid cyst of, 144
tissue anomalies and their relationship to some tumors of, 851
Ovulation, effect of injection of urine from pregnant mammals on ovulation in rabbit, 470

Paget's disease of nipple: See under Breast
Pancreas, action of epinephrine in depancreatized animals, 141
cysts, 2 cases of, 835
hyperinsulinism from B-cell adenoma of, 282
morphology of so-called argentaffine cells in, 1019
sarcoma of, primary, 309
Pancreatitis, complicating mumps, 120
hemorrhagic, 288
Pappenheimer, A. M.: Nutritional encephalomalacia in chicks, 134
Nutritional muscular dystrophy in guinea-pigs and rabbits, 140
Paraffin sections, use of butyl alcohol in preparation of, *599
sickness, pathologic anatomy of, 125
Parasitus, etiology of cancer; tissue transplantation and, *983
Parathyroidism, availability of calcium reserve of spongy trabeculae and compact bone in experimental hyperparathyroidism, 142
Parathyroids, experimental chronic hyperparathyroidism leading to ostitis fibrosa, 283
sites of decalcification and of bone lesions in experimental hyperparathyroidism, *715
Paratyphoid, variability, not type specificity, in group of paratyphoid-food poisoning bacteria, 659
Parkinsonism, encephalitic, idiopathic and arteriosclerotic, clinicopathologic study, 642
Parotitis, pancreatitis complicating mumps, 120
Parsons, E.: Hemorrhage and "shock" in traumatized limbs; changes in total, free and bound water of blood and muscle, *869
Hemorrhage and "shock" in traumatized limbs; total, free and bound water changes of blood and muscle, 144
Paternity, heredity of agglutinogens M and N of Landsteiner and Levine with particular reference to determination of, 679
Pathology, doctorates in bacteriology and pathology granted by American universities, 1930-1931, 468
Pellagra: etiology (modern theories) and pathologic anatomy, *970
is rat dermatitis consequent on vitamin B_2 (G) deficiency true pellagra? 1016
Pellagra—Continued
secondary to benign and carcinomatous lesions and to dysfunction of gastro-intestinal tract, 281
Periarteritis nodosa complicated by fatal intrapericardial hemorrhage, report of case, *521
Pericardium, diverticulum, 834
periarteritis nodosa complicated by fatal intrapericardial hemorrhage, report of a case, *521
sarcoma of, primary, 852, 853
Peritoneum, sugar content of pleural and peritoneal exudates, 127
Peritonitis, bile, chemical changes in blood of dog in, 482
fat necrosis in bile peritonitis: experimental study, *70
septic, in man due to Bacillus sordelli; report of case, *245
Perla, D.: Bartonella muris anemia; compensatory phenomena following splenectomy in adult albino rat, 676
Bartonella muris anemia; pathologic changes during acute anemia, 675
Perlstein, M. A.: Effects of intrathecal administration of mercurochrome-220 soluble and of metaphen, *729
Petersen, W. F.: Menstruation and focal reactions, 141
Renal denervation; effect of snake venom; effect of repeated chillings, 142
Phaeochromocytoma, malignant, of adrenals, 850
Phagocytosis and spleen, 496
of colloid bodies in living body and in tissue cultures, 639
mechanism of action of bacteriotropin: relative degree of phagocytosis by polymorphonuclear leukocytes and by monocytes, 137
promotion of phagocytosis by various chemical substances, 131
relation of cell granules to phagocytosis of bacteria, 843
Phosphorus and calcium in brain in different conditions, 482
poisoning, acute, 310
Pick's disease: See Brain, atrophy
Pinealomas, 849
Pituitary Body; origin of hypophyseal cachexia (Simmond's disease) 637
rôle of anterior lobe of pituitary gland in growth: with reference to teeth and maxillae, *37
teratoid tumor of hypophyseal region, 852
tumors, extrasellar, 4 cases, 835
Pituitary Extracts, effects of posterior pituitary extracts on basal metabolism, 281
influence of female sex hormone and anterior hypophyseal hormone on structure of hypophysis, 638
Placenta, transmission of sensitization from mother to child, 494
transplacental tuberculous infection, 294
Plague, immunity in experimental plague, 304
pneumonic, in Tunis, 294
pulverized plague vaccine via respiratory tract, 305
Plasma Cells: See under Cells
Plethora, 638
Pleura, absorption from pleural cavity of dogs, 288
apical pleural indurations, 834
effect on pulmonary epithelium by intrapleural injections of electrolytes, 471
morbid anatomy of malignant disease of mediastinal glands, lungs and pleura, 644
sugar content of pleural and peritoneal exudates, 127
tooth in pleural cavity, 288
Pneumococcus, action of a bacterial enzyme on type III pneumococcus, 1028
experimental dermal pneumococcus infection in rabbit, 1028
heterophile antigen in, 492
immunity in lungs of rabbits following immunization with, 302
immunological relationships among, 297
immunology of, 491

Pneumococcus—Continued
infection, epidemiology of, 292
intrabronchial insufflation of pneumococci in rabbits, 652
intracutaneous pneumococcus injection, 655
intratracheal inoculation of monkeys with, 652
local skin reactivity (Shwartzman's reaction) to pneumococcus "filtrates," 655
metabolism of S and R forms of, 292
passive immunity with pneumococcus III, 304
specific and nonspecific polysaccharides of type IV pneumococcus, 298
Pneumoconiosis, action of animal tissues on silicious spicules of fresh water sponge, 125
Pneumonia, experimental tuberculin pneumonia, 1025
interstitial, 835
pneumococcus, mouse protection tests for antibody in, 129
Polayes, S. H.: Psittacosis; postmortem examination of case, including studies of spinal cord, 319
Poliomyelitis, 293
active immunization in monkeys, 654
concentration of protective substance in antipoliomyelitis, serum, 302
effect of filtration and of various constituents of tissue on virus of, 137
epidemic, protective inoculation against, 844
epidemiology of, 293
experimental, 652
immunization with mixtures of poliomyelitis virus and aluminum hydroxide, 297
immunological differences between strains of poliomyelitic virus, 656
nervous lesions of subacute or chronic experimental poliomyelitis, 643
occurrence of intranuclear inclusions in nerve cells in, 839
potent antipoliomyelitic horse serum concentrate, 298
protection of monkey by antipoliomyelitic serum, 129
Pollen extracts, skin reactions to pollen extracts in rabbits, 131
Polyarteritis nodosa: See Periarteritis nodosa
Polyblasts, so-called, origin of, 838
Polypi, development of carcinoma from polypi of colon, 138
Porencephalus, malformation of brain with hydrocephalus and, 836
Postmortems: See Autopsies
Potassium chlorate poisoning, 506
Precipitins, experimental studies on formation of, 659
in diphtheria immune serum, 2 kinds, 498
specificness of hemoglobin precipitins, 656
Pregnancy, action of blood of pregnant women on frog larvae, 831
Aschheim-Zondek test for, modified, 854
effect of injection of urine from pregnant mammals on ovulation in rabbit, 470
grave anemia in pregnant women from benzene intoxication, 310
hydrogen-ion concentration and acid-base equilibrium in, 482
Prize, Monaco prize for work in bacteriology to Martin R. Veillon, 467
Prostate, incidence of cancer of bladder and prostate in certain occupations, 663
Protein, anaphylactogenic action of protein from filtrates of acid-fast bacteria, 299
congenital protein hypersensitiveness in 2 generations, 298
effects of ultraviolet rays, radium and x-rays on, *533
Protozoa, effect of toxins and venoms on, 486
resistance of protozoa to crotalus and cobra venoms, 290
Pseudohermaphroditismus: See Hermaphroditism
Psittacosis, 1029
postmortem examination of case, including studies on spinal cord, 319
virus of, 489
Pylorus, rôle of pyloric sphincter in control of gastric acidity, 1016
Pyocyanine, an accessory respiratory enzyme, 635
Rabies, intensifying prophylaxis of, 660
splenic lesions in canine rabies, 488
Rachitis: See Rickets
Radiation: See also Radium; Roentgen Rays; Ultraviolet Rays
mitogentic, 472
Radio, plant and animal experiments with a short wave radio oscillator, 473
Radioactive deposits, occurrence of malignant growth in persons with, 511
Radium, chronic industrial radium poisoning in Joachimsthal, 665
effects of ultraviolet rays, radium and x-rays on proteins, *533
Radon, effects on blood sugar and its distribution between cells and plasma, 474
Rand, G.: Congenital diaphragmatic hernia associated with accessory lung, 146
Rape and deformity of male sexual organs, 314
Rascoff, H.: Congenital tracheo-esophageal fistula caused by an aberrant ductus arteriosus, 508
Ratcliffe, H. L.: Comparative study of amebiasis in man, monkeys and cats, with special reference to formation of early lesions, 327
Raynaud's disease, micro-injection studies of capillary blood pressure in, 284
Rectum, case of retrorectal lipoma, 646
Reflexes, rôle of nervous system and of conditional reflexes in immunity, 496
Reimann, S. P.: Hyperplasia of skin of rat and mouse from sulphydryl, 135
Respiratory tract, bacterial flora of respiratory system of normal dogs, 487
sudden death due to syphilis of respiratory organs, 2 cases, 312
Reticulo-Endothelial System, appearance of histiocytes, in peripheral blood, 477
experimental studies on reticulo-endothelial system: influence of india ink on ratio of urea in blood, *180
Reticulosis, *773
acute aleukemic, 838
Retina, angioreticuloxanthoma of retina (von Hippel and Lindau disease), 851
Rewbridge, A. G.: Fat necrosis in bile peritonitis; experimental study, *70
Rhabdomyoma, congenital, of heart, 500
Rheumatic Fever, acute, experiments bearing on, 656
involvement of coronary arteries in, 120
Rheumatism, acquired rheumatic pulmonic stenosis and insufficiency, 476
Rickets, calcium content of striated muscle of rachitic animals, 126
experimental rickets and calcification of dentin, *358
generalized osteitis fibrosa and its relation to osteomalacia and rickets, 1045
Rickettsia orientalis, cause of Tsutsugamushi disease, 843
Rieckenberg reaction, 305
Robertson, H. E.: Development of carcinoma from polypi of colon, 138
Robinson, G. H.: Cytologic reactions in experimental monilial abscesses, 136
Robinson, W.: Hemorrhage and "shock" in traumatized limbs; changes in total, free and bound water of blood and muscle, *869
Hemorrhage and "shock" in traumatized limbs; total, free and bound water changes of blood and muscle, 144
Roentgen Rays, aplasia of marrow and fatal intoxication in dogs produced by roentgen radiation of all bones, 119
carcinoma and sarcoma, 849
combined effects of colloidal silver and highly filtered roentgen radiation upon hematopoietic system in dogs, 119
effects of ultraviolet rays, radium and x-rays on proteins, *533
influence of x-ray lesions of intestinal mucosa on absorption of glucose and other sugars, 471
shadows produced by lead in x-ray pictures of growing skeleton, 287

Roentgen Rays—Continued
skin reactions produced by alternations of heat and x-ray at various time intervals, 118
tumors developing after x-ray treatment of bone and joint tuberculosis, 851
Rosenthal, A. H.: Congenital atresia of esophagus with tracheo-esophageal fistula, report of 8 cases, *756
Rosenthal, L.: Bartonella anemia in nonsplenectomized rats, *405
Rosenthal, S. R.: Isolated giant growth of branch of pulmonary artery associated with congenital bronchiectasis, report of case, *387
Uremic dermatitis, 143
Ryder, W. B.: Experimental rickets and calcification of dentin, *358

Saliva, diphtheria antitoxin in, 494
Salivary Gland, cartilage in, *31
histological study of, 662
Salmonella: See under Bacteria
Sands, I. J.: Lesions of brain in influenza, 682
Sanford, A. H.: Collective review of recent books on clinical pathology, 857
aqueous extract of suprarenal cortex on growth of, 847
Sarcoma, influence of aqueous extract of suprarenal cortex on growth of, 847
influence of suprarenalin (epinephrin) on growth of carcinoma and sarcoma in animals, 307
neurogenic, 1038
of esophagus, 1040
osteogenic sarcoma-like tumor of metacarpal bone, 678
primary, of pancreas, 309
primary, of pericardium, 852, 853
roentgen carcinoma and sarcoma, 849
stimulating effect of arsenic on chicken sarcoma, 502
Scalp, mixed tumor of, 145
Scarlet fever, experimental, 488
experimental scarlatinal nephritis in dog, *572
laboratory infection with, 295
Schilder's Disease: See Encephalitis periaxialis diffusa
Schüller disease, xanthomatosis with cranial defects, 832
Scleroderma, pathology of, 123
Sclerosis, multiple, pathogenesis of, 830
Secretions, Internal: See Endocrine Glands
Semenov, H.: Experimental arteritis and arteriosclerosis associated with streptococcal inoculations, *924
Senile plaques, origin and formation of, 643
Serodiagnosis, specificity of immune serum and serum diagnosis of bacteria, 497
"zoning" phenomenon in complement fixation, 132
Serology, occurrence of serologic factors M and N in Japanese, 1038
Serum, toxicity of certain normal sera for guinea-pig, 119
Sex hormone, influence of female sex hormone and anterior hypophyseal hormone on structure of hypophysis, 638
Shapiro, P. F.: Generalized actinomycosis (pyemic form), report of case, *397
Sherman, I.: Experimental studies on reticuloendothelial system; influence of india ink on ratio of urea in blood, *180
Shock, hemorrhage and "shock" in traumatized limbs: changes in total, free and bound water of blood and muscle, *869
Shwartzman's reaction; local skin reactivity (Shwartzman's reaction) to pneumococcus "filtrate," 655
Siebert, W. J.: Homoiotransplantation and heterotransplantation in guinea-pig; effects of graded degrees of heat on cartilage and on thyroid gland, *590
Silicosis: See Pneumoconiosis
Silver ammonium carbonate, supravital staining with, 506
combined effects of colloidal silver and highly filtered roentgen radiation upon hematopoietic system in dogs, 119
effects of intravenous injection of colloidal silver upon hematopoietic system in dogs, 118
Simmond's disease: See Pituitary Body
Skeleton, repair in vitro of embryonic skeletal rudiments after experimental injury, 644
Skin as an immunological organ, 1035
diseases, ultraviruses inducing neurotropic ectodermoses, 490
effect of homologous macerated skin on regeneration of epidermis, *794
hyperplasia of skin of rat and mouse from sulphydryl, 135
hypersensitiveness in guinea-pigs infected with Brucella abortus, 131
neutralization of skin reactions by antimeningococcus serums, 301
reaction to agar after injection of meningococcus filtrate, 131
reactions produced by alternations of heat and x-ray at various time intervals, 118
reactions to pollen extracts in rabbits, 131
reactivity, local skin reactivity to bacterial filtrates: passive immunity, 654
reactivity, local skin reactivity (Shwartzman's reaction) to pneumococcus "filtrates," 655
sugar content of skin and muscle in diabetic and non-diabetic persons, 126
Smallpox, immunization against vaccinia by non-infective mixtures of virus and immune serum, 130
Vaccination, Encephalomyelitis following: See under Encephalomyelitis
vaccination; spontaneous immunization of rabbits to vaccine virus, 493
vaccination; vaccinia; susceptibility of mice and immunologic studies, 491
vaccine, survival of vaccine virus separted from living host cells by collodion membranes, 291
variola-vaccinia flocculation, 659
Smith, K. G.: Experimental arteritis and arteriosclerosis associated with streptococcal inoculations, *924
Smith, L. W.: Case of congenital absence of 1 kidney, with urethrorectal fistula, urethral stone and concomitant measles, 315
Snake venom; renal denervation; effect of snake venom; effect of repeated chillings, 142
Societies, Division of Medical Sciences of National Research Council elects officers, 275
International Association for Geographic Pathology, 467
International Congress of Comparative Pathology, 275
Society Transactions:
American Society for Experimental Pathology, 134
Chicago Pathological Society, 143
New York Pathological Society, 315, 508, 668
Pathological Society of Philadelphia, 322, 1045
Sodium hydroxide poisoning, histopathology of, 314
salicylate, detoxifying and disinfecting properties of, 132
Soil and drinking water in relationship to malignant tumors in Italy, 309
Spiegel-Adolf, M.: Effect of ultraviolet rays, radium and x-rays on proteins, *533
Spinal cord, cerebellar hemangioblastomas with incidental changes of, 122
intramedullary tumors of, 306
Spine, assimilation of atlas and compression of medulla; clinical significance and pathology of torticollis and localized chronic arthritis deformans of spine, report of case, *333
Spirochaeta pallidum, antigenic peculiarities of strains of, 844
Spleen and phagocytosis, 496
compensatory hypertrophy of, 282
hemendothelioma of spleen and bone marrow, 501
lipoid cell hyperplasia of, 1020
proteolytic activity of, 1021
rôle of spleen in metabolism, 639
Splenectomy, Bartonella anemia an nonsplenectomized rats, *405
Splenomegaly and hepatic enlargement in hereditary hemorrhagic telangiectasia, 116
importance of, in essential thrombopenia, 832

Sponge, action of animal tissues on silicious spicules of freshwater sponge, 125
Sporangia, occurrence of unidentified sporangia in tonsil crypts, *33
Sputum, tuberculous, effects on guinea-pigs of filtrates of, 653
Stains and Staining, modification of Cajal's gold sublimate method for demonstrating macroglia cells in formaldehyde-fixed material, 667
supravital staining with silver ammonium carbonate, 506
Staphylococcus aureus, non-specific local cutaneous immunity to, 297
filtrates, immunization with, 302
toxin anatoxin and antitoxin, 658
Status lymphaticus: See Lymphatism
Steinberg, B.: Method for examining appendix, *598
Stephenson, M.: Some problems of bacterial respiration, 1045
Stevens, H. C.: A new brain slicer, *90
Stewart, H. L.: Asbestosis; report of 2 cases, *909
Stewart, J. D., Jr.: Myoma of esophagus with associated diverticula; report of 2 instances, *77
Stomach, acidity, rôle of pyloric sphincter in control of, 1016
formation of giant folds in mucosa of, 646
healing of gastric mucous membrane in rabbits after its surgical removal, *85
Streptococcus, action of congo red on streptococcal and B. welchii hemolysin, 658
dissociation of hemolytic streptococci, 1031
epidemicus, toxins of, 1037
experimental arteritis and arteriosclerosis associated with streptococcal inoculation, *924
hemolytic and nonhemolytic, chemical differentiation of, 841
hemolytic, intravenous vaccination with, 1035
local skin reactivity to streptococcus hemolyticus-scarlatinae, 132
otitis media and mastoiditis due to streptococcus epidemicus, 1031
relation of streptococci to filtrable virus of epizootic encephalitis of fox, 488
septicemia with vascular lesions, 485
sporadic cellulitis and abscess due to streptococcus epidemicus, 1031
tonsillectomy in carriers of streptococcus epidemicus, 1031
Strumia, M. M.: Hodgkin's disease of unusual duration and extension, 323
Mechanism of action of bacterio-tropin; relative degree of phagocytosis by polymorphonuclear leukocytes and by monocytes, 137
Strychnine, detoxicating function of liver with special reference to, 281
Sugar content of pleural and peritoneal exudates, 127
content of skin and muscle in diabetic and non-diabetic persons, 126
Suicide or apoplexy, 1043
Sullivan, F. L.: Immunity of tissues: conditions influencing fixation and destruction of bacteria in tissues, 138
Sulphur, nonprotein sulphur in blood, plasma and serum and glutathione sulphur in blood, 1022
relation of sulphur to experimental anemia, 140
Sulphydryl, hyperplasia of skin of rat and mouse from, 135
Suprarenalectomy, agglutinin production in suprarenalectomized rats, 1037
Suprarenals and tumor growth, 847
carcinoma of suprarenal cortex with unusual metastases, 319
changes in blood and renal function after removal of celiac and superior mesenteric ganglions and left adrenal gland, 285
contraction of suprarenal glands and atrophy of testes, 836
extract; influence of aqueous extract of suprarenal cortex on growth of carcinoma, sarcoma and melanoma in animals, 847
extract, influence on growth of carcinoma, sarcoma and melanoma, 847
hyperplasia; pseudohermaphroditismus masculinus externus associated with suprarenal hyperplasia and vascular hypertension, report of case, *543
Suprarenals—Continued
malignant phaeochromocytoma of, 850
preparation; complications following endocardiac injections, 1044
preparations; action of epinephrine in depancreatized animals, 141
preparations; influence of suprarenalin on growth of carcinoma and sarcoma, 307
Sweat, diphtheria antitoxin in human sweat, 1036
Syphilis, correlation of roentgenologic picture with anatomic changes in congenital osseous syphilis, 287
modification of serodiagnosis of syphilis with inactivated serum, 659
nonsyphilitic aortic valve deformity, *889
of respiratory organs, 2 cases of sudden death due to, 312
Syphilis, precipitation tests for diagnosis of, value of, 856
serology of, 506
theoretical considerations in connection with a cold incubation complement fixation test, 492
ultramicroscopic precipitation reaction in, 301

Tapeworm, native infestation with diphyllobothrium latum (fish tapeworm), 839
Tar Cancer: See under Cancer
Teeth, rôle of anterior lobe of pituitary gland in growth with reference to teeth and maxillae, *37
Telangiectasia, splenomegaly and hepatic enlargement in, 116
Temperatures, effect of supranormal temperatures on tissue cultures, 1016
tumor of brain with disturbance in temperature regulation, 116
variations in temperature of healthy and tuberculous birds, 135
Teratoid tumor of hypophyseal region, 852
Teratomas of mediastinum, 2 cases, 480
Testes, atrophy of, and contraction of suprarenal, 836
cellular reaction to infection with tubercle bacilli; experiments on testes of normal and immunized guinea-pigs, 135
extract, influence on intradermal spread of injected fluids and particles, 117
extract, effect on red cells, 635
extract, effect on tissue permeability, 472
Tetanus, mechanism of toxin-antitoxin reactions, 1036
spores of clostridium tetani, 1030
Thatcher, H. S.: Pellagra; etiology (modern theories) and pathologic anatomy, *970
Thompson, R.: Etiology of postvaccinial encephalomyelitis, *601
Thorax, cysts, 480
nonrachitic soft chest and flat head, a new syndrome, 475
Thrombo-angiitis obliterans, 481
obliterans, chemistry of blood in, 1021
Thrombocytosis, reactions of bone marrow in experimentally induced thrombocytosis, *153
Thrombopenia, essential, 837
essential, importance of splenomegaly in, 832
Thrombosis, progressive thrombosis of pulmonary artery, 477
structure of young blood platelet thrombi, 123
Thyroid, cancer of, 316
effects of graded degrees of heat on cartilage and on thyroid gland, *590
experimental hyperthyroidism in guinea-pigs and rabbits, 471
heterotransplantation of thyroid gland, *590
malignant mixed tumors with skeletal muscle fibers, 846
morbid anatomy of heart in hyperthyroidism, 478
Tibia, adamantinoma of, primary, 501, 1042
Tick-bite fever, agglutinins of tick-bite fever and sporadic typhus in southern Africa, 657
Ticks as vectors of typhus fever, 1027
Tissue culture, bactericidal substances in, 1038
culture, effect of supranormal temperatures on, 1016
culture, influence of chemical substances on cells in tissue culture, 1018
culture significance of, for pathologic physiology, 1017

Tissue—Continued
immunity; conditions influencing fixation and destruction of bacteria in tissues, 138
permeability, effect of testicular extract upon, 472
Tonsillectomy in carriers of streptococcus epidemicus, 1031
Tonsils, occurrence of unidentified sporangia in tonsil crypts, *33
Torticollis, assimilation of atlas and compression of medulla: clinical significance and pathology of torticollis and localized chronic arthritis deformans of spine, report of case, *333
Toxin, surface phenomena in reaction of toxin and antitoxin, 495
Toxin-antitoxin union, 301
Trachea, infection of rabbits with tubercle bacillus; studies on defensive and metabolic apparatus of lungs, *689
Tracheo-esophageal fistula with congenital atresia of esophagus, report of 8 cases, *756
Trachoma, bacterium granulosis in relation to trachoma; pathogenicity for various monkeys and apes, 1033
experimental, effect of secondary infections on, 292
transmission and cultivation experiments with, 1027
Transplantation, homoiotransplantation and heterotransplantation in guinea-pig; effects of graded degrees of heat on cartilage and on thyroid gland, *590
individuality differentials in strains of inbred rats, *203
Trauma, hemorrhage and "shock" in traumatized limbs: changes in total, free and bound water of blood and muscle, *869
hemorrhage and "shock" in traumatized limbs; total, free and bound water changes of blood and muscle, 144
Trichinella spiralis, human trichinella spiralis infections and Bachman precipitin test, 296
Trypan blue, effect of injection of trypan blue on rate of sedimentation of erythrocytes in inflammation, *429
Tsutsugamushi disease, Rickettsia orientalis as cause of, 843
Tubercle Bacillus, avian tubercle bacillus infection, with special reference to mammals and to man: its reported association with Hodgkin's disease, *253
bacteriologic investigations of strain isolated from lungs and lymph nodes of child vaccinated with B C G, 499
cellular reaction to infection with tubercle bacilli; experiments on testes of normal and immunized guinea-pigs, 135
commemoration of discovery of, 1014
cultivation from blood according to Löwenstein, 841
developmental cycle of, 1034
early cellular reaction to; comparison of this reaction in normal and tuberculous guinea-pigs and guinea-pigs immunized with dead bacilli, *956
early cellular reaction to human type tubercle bacilli in lung of rabbit, 143
effect on growth of tubercle bacillus of modification of amount of glycerol in tissues, 1024
egg yolk agar medium in, 487
filtrability and polymorphism of, 1033
filtrability of, 295
filtrable forms of, 1034
Hohn's medium and thick seeding of sediment, 842
in blood in laryngeal tuberculosis, 841
in blood in various diseases, 294
in children with erythema nodosum, 1023
infection of rabbits by way of trachea; studies on defensive and metabolic apparatus of lungs, *689
method for counting dead and living tubercle bacilli in sputum, 856
morphologic variation of, 1034
peroral infection of guinea-pigs in, 841
secondary colonies, autolysis and lysis in cultures of, 842
variability of, 1034
Tubercle Bacillus—Continued
variations of tubercle bacillus in form and action, 1034
various forms of, 1034
Tuberculin, anaphylactic and tuberculin types of hypersensitiveness, 492
chemical composition of active principle of, 1024
experimental tuberculin pneumonia, 1025
production and purification of, 845
sensitivity in rats, 302
sensitivity of guinea-pigs under acidotic diet, 132
specificity of avian tuberculin reactions, 1037
types, anaphylactic and tuberculin types of hypersensitiveness, 299
Tuberculosis, accumulation of iron in tuberculous areas, 1027
acute generalized tuberculosis without typical tubercles, 485
anatomical characteristics of tuberculosis in Jamaica, 1024
avian, pathological reaction of dogs to, 290
B C G, action of, 845
B C G, bacteriologic investigations of strain of tubercle bacilli isolated from lungs and lymph nodes of child vaccinated with B C G, 499
B C G, criticism of B. Lange's studies concerning causes of Lübeck disaster, 660
B C G, influence of Calmette-Guérin bacilli on animals infected with tuberculosis, 304
B C G vaccine, protective action of, 845
B C G vaccination, causes of illness in infants following vaccination with Calmette-Guérin bacilli in Lübeck, 295
bacteriologic diagnosis of, 133
bronchial glands, latent and occult tuberculosis of, 489
chemical view of pathogenesis of, 1023
complement fixation and serum lability test in, 133
effects on guinea-pigs of filtrates of tuberculous sputum 653
experimental, 136, 842
immunity in, 660
immunizing rabbits against, attempts to, 498
inoculation of tuberculous material into lymphatic ganglions, 133
late tuberculous primary complex in adults, 841
lipolytic activity of tissues of tuberculous guinea-pigs, 1017
mesenteric lymph nodes of children 484
monocytes in tuberculous animals, 1025
multiple pulmonary abscesses simulating, 485
pathologic anatomy in children, 833
plasma protein, red-cell sedimentation and protection against, 659
pulmonary, a tuberculous primary focus with atypical histologic structure, 842
silicic acid content of blood in tuberculous patients, significance of, 843
spontaneous, in domestic animals with reference to Ranke's teaching, 840
studies on tuberculous liquefaction, 840
susceptibility of gopher to, 290
transplacental tuberculous infection, 294
tumors developing after x-ray treatment of bone and joint, 851
variations in temperature of healthy and tuberculous birds, 135
virus, filtrable elements of, 842
Tuberculotoxins, 493
Tularemia, tularemic leptomeningitis; report of a case, *917
Tumors, collision, 1041
developing after x-ray treatment of bone and joint tuberculosis, 851
experimental tar tumors in white rat, 850
growth of tumors in tissue without nerves, 503
immunity, 499
immunity: effects of eu- and pseudo-globulin fractions of anti-cancer sera on tissue cultures, 502
immunity to transplantable rat tumors, 848
in captive primates (giant cell tumor in a Chacma baboon), 662
intramedullary tumors of spinal cord, 306
metastasis of "benign" giant-cell tumor of bone, 502
mixed, of scalp, 145

Tumors—Continued
occupational neoplastic diseases, 847
of neuromyo-arterial glomus (Masson), 852
of posterior cranial fossa causing visual hallucinations, 289
oncogenic factor in blood and organs of animals with inoculation tumors, 309
purified (protein-free) virus of chicken tumor No. 1, 660
salting out of agent of chicken tumor with globulin of filtrates, 306
soil and drinking water in relationship to malignant tumors in Italy, 309
spontaneous, statistical study of occurrence in large colony of rats, 846
tissue anomalies and their relationship to some tumors of ovaries, 851
tissue, effect of irradiated ergosterol and increased calcium on growth of, 308
transplantable, influence of vital staining on induced resistance to growth of, 501
Twins, mongolism in one of, 116
Typhoid bacilli, effects of other bacteria on resistance to infection with, 844
bacillus, gas-forming variants of, 841
Typhus, active immunization against typhus fever with formalinized virus, 296
active immunization against Mexican typhus fever with dead virus, 297.
agglutinins of tick-bite fever and sporadic typhus in southern Africa, 657
endemic typhus in Virginia, 290
reduction of resistance to typhus virus by diet deficiency, 291
serological varieties of, 303
silent typhus infection, 840
ticks as vectors of, 1027

Udders, bacterial growth in udders of cows, 489
Ultraviolet rays, effects of ultraviolet rays, radium and x-rays on proteins, *533
Ultraviruses: See under Viruses
Undulant fever, epidemiology of, 1031
in Azerbaidjan, 293
Urea, experimental studies on reticulo-endothelial system: influence of india ink on ratio of urea in blood, *180
influence of intravenous injection of urea on exchange of substances between blood and tissues, 831
Uremia, dermatitis in, 143
Ureter, primary malignant tumor of, 662
Urethral calculi, case of congenital absence of 1 kidney, with urethrorectal fistula, urethral stone and concomitant measles, 315
Urine, acetone, quantitative determination of acetone with Pulfrich's photometer, 855
alkalinity and phosphate content of morning urine, 482
antibodies in urine after injection of antitoxic globulin, 298
effect of injection of urine from pregnant mammals on ovulation in rabbit, 470
insulin in, occurrence of, 1022
lead in feces and, 649
loss of diphtheria antitoxin in, 493
Urobilinogen, standardization of method based on mesobilirubinogen (H. Fischer) for estimation of urobilinogen, 854
Uterus, cancer, grading of carcinoma of cervix uteri as checked at autopsy, *783

Vaccine, advaccine (antidenatured vaccine), 497
Vance, B. M.: Periarteritis nodosa complicated by fatal intrapericardial hemorrhage; report of case, *521
Vanderveer, H. L.: Hypervitaminosis D and arteriosclerosis, *941
Varicella: See Chickenpox
Vasa deferentia, calcification of vasa deferentia and ampullae, 645
Vater's ampulla, calcification of vasa deferentia and ampullae, 645
de Vecchi, B.: Endocarditic process in childhood, *49
Veins, direct communications between arteries and veins in rabbitt's ear, 285
Venoms, resistance of protozoa to crotalus and cobra venoms, 290
Vibrios, vibrionic enteritis in calves, 1026
vibrio jejuni, n. sp. associated with intestinal
Vibrios, vibrionic enteritis in calves, 1026
Viruses, new collodion membranes for bacteriological use, 855
ultraviruses inducing neurotropic ectodermoses, 490
Vision, tumor of posterior cranial fossa causing visual hallucinations, 289
Vitamin B_2, is rat dermatitis consequent on vitamin B_2 (G) deficiency true pellagra? 1016
D, hypervitaminosis D and arteriosclerosis, *941
Vorwald, A. J.: Cellular reaction to infection with tubercle bacilli; experiments on testes of normal and immunized guinea-pigs, 135
Early cellular reaction to human type tubercle bacilli in lung of rabbit, 143
Early cellular reaction to tubercle bacilli; comparison of this reaction in normal and tuberculous guinea-pigs and guinea-pigs immunized with dead bacilli, *956

Walker, M. A.: Healing of gastric mucous membrane in rabbits after its surgical removal, *85
Warren, S.: Cancer of thyroid, 316
Grading of carcinoma of cervix uteri as checked at autopsy, *783
Warthin, A. S.: Occurrence of unidentified sporangia in tonsil crypts, *33
Wassermann reaction, relation between Wassermann reaction and blood groups, 499
specific Wassermann reaction in hepatic distomiasis, 496
with fluids of tissues, 496
Water balance, significance of water balance in epilepsy, 139
experimental studies of high plunge into, 312
hemorrhage and "shock" in traumatized limbs: changes in total, free and bound water of blood and muscle, *869
soil and drinking water in relationship to malignant tumors in Italy, 309
Weidman, F. D.: Pulmonary "pseudo-actinomycosis" (chromoblastomycosis?) in a capybara due to a hyphomycete producing ray fungi in tissue, 326
Weller, C. V.: Aldred Scott Warthin, 276
Whooping cough, blood picture in, 1023
Wiener, A. S.: Heredity of agglutinogens M and N of Landsteiner and Levine with particular reference to determination of paternity, 679
Wilbur, D. L.: Normal renal glomerulus of man, histologic consideration, *413
Wilson, F. N.: Character of excitation wave in auricular muscle, 134
Wolfstein, M.: Malignant hemangioma of lung with multiple visceral foci, report of case, *562
Wounds, effect of homologous macerated skin on regeneration of epidermis, *794
gunshot, spattering of tissue from, 664
Wry-neck: See Torticollis

Xanthoma, angioreticuloxanthoma of retina (von Hippel and Lindau disease), 851
xanthomatosis with cranial defects (Schüller's disease), 832
Xanthoprotein, lipoids and dextrose in blood serum of persons with cancer, 309

Yellow fever, complement fixation in yellow fever in monkey and in man, 295
myocardium in, 636

Zohman, B. L.: Bartonella anemia in nonsplenectomized rats, *405
"Zoning" phenomenon in complement fixation, 132

THE ARCHIVES OF PATHOLOGY is published by the American Medical Association as a medium to advance the science of pathology in the United States and for the promotion of research and observation in this field.

Manuscripts for publication, books for review, and correspondence relating to contributions should be sent to Dr. Ludvig Hektoen, 637 South Wood Street, Chicago, or to any other member of the Editorial Board. Communications regarding subscriptions, reprints, etc., should be addressed, ARCHIVES OF PATHOLOGY, American Medical Association, 535 North Dearborn Street, Chicago.

Articles are accepted for publication on the condition that they are contributed solely to the ARCHIVES OF PATHOLOGY. Manuscripts must be typewritten, preferably double spaced, and the original copy should be submitted. Zinc etchings and halftones of illustrations will be supplied by the publisher when the original illustrations warrant.

Footnotes and bibliographies should conform to the style of the Quarterly Cumulative Index Medicus, published by the American Medical Association. This requires, in order given: name of author, title of article, name of periodical, with volume, page, month—day of month if weekly—and year. A complete list of abbreviations for standard periodicals, together with a full discussion of the style of the A. M. A. publications, appears in The Art and Practice of Medical Writing, a comprehensive book on the preparation of medical manuscripts, published by the American Medical Association. Price, $1.50.

Matter appearing in the ARCHIVES OF PATHOLOGY is covered by copyright, but, as a rule, no objection will be made to the reproduction in reputable medical journals if proper credit is given. However, the reproduction for commercial purposes of articles appearing in the ARCHIVES OF PATHOLOGY, or in any of the other publications issued by the Association, will not be permitted.

Authors will receive one hundred reprints free; additional reprints can be obtained at cost.

The ARCHIVES OF PATHOLOGY is published monthly. Annual subscription price (two volumes): Domestic, $6.00; Canadian, $6.40; foreign, $6.75, including postage. Single copies, 75 cents, postpaid.

Checks, etc., should be made payable to the AMERICAN MEDICAL ASSOCIATION.

OTHER PERIODICAL PUBLICATIONS
of the American Medical Association

THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION—Weekly. Covers all the medical activities and matters of general medical interest. Illustrated. Annual subscription price (two volumes): Domestic, $7.00; Canadian, $8.00; foreign, $9.50. Single copies, 25 cents.

ARCHIVES OF INTERNAL MEDICINE—Monthly. Devoted to the publication of advanced, original clinical and laboratory investigations in internal medicine. Illustrated. Annual subscription price (two volumes): Domestic, $5.00; Canadian, $5.40; foreign, $5.75. Single copies, 75 cents.

AMERICAN JOURNAL OF DISEASES OF CHILDREN—Monthly. Presents pediatrics as a medical science and as a social problem. It includes carefully prepared collective abstracts based on current pediatric literature, abstracts from foreign and domestic literature, book reviews, society transactions, etc. Illustrated. Annual subscription price (two volumes): Domestic, $6.00; Canadian, $6.40; foreign, $6.75. Single copies, 85 cents.

ARCHIVES OF NEUROLOGY AND PSYCHIATRY—Monthly. A medium for the presentation of original articles on nervous and mental diseases, with abstracts from foreign and domestic literature, book reviews, society transactions, etc. Illustrated. Annual subscription price (two volumes): Domestic, $5.00; Canadian, $5.40; foreign, $5.75. Single copies, 85 cents.

ARCHIVES OF DERMATOLOGY AND SYPHILOLOGY—Monthly. Devoted to advancing the knowledge of and progress in cutaneous diseases and syphilis. Publishes original contributions and the abstracts of the literature on these subjects, transactions of the important dermatological societies, book reviews, etc. Illustrated. Annual subscription price (two volumes): Domestic, $6.00; Canadian, $6.40; foreign, $6.75. Single copies, 85 cents.

ARCHIVES OF SURGERY—Monthly. Devoted largely to the investigation and ablest phases of surgery, with monthly reviews on orthopedic and urologic surgery. Well illustrated. Annual subscription price (two volumes): Domestic, $7.00; Canadian, $8.40; foreign, $8.75. Single copies, 85 cents.

ARCHIVES OF OTOLARYNGOLOGY—Monthly. The medium for the presentation of original articles on diseases of the ear, nose and throat, with abstracts from foreign and domestic literature, book reviews, the transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): Domestic, $6.00; Canadian, $6.40; foreign, $6.75. Single copies, 75 cents.

ARCHIVES OF OPHTHALMOLOGY—Monthly. Includes original articles on diseases of the eye, abstracts from foreign and domestic literature, book reviews, transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): Domestic, $6.00; Canadian, $6.40; foreign, $6.75. Single copies, 85 cents.

QUARTERLY CUMULATIVE INDEX MEDICUS—Quarterly. A complete subject and author index to the worth-while current medical literature of the world. Issued four times a year. Second and fourth volumes bound for permanent reference. Subscription price, calendar year: Domestic, $10.00; Canadian, $10.56; foreign, $11.05.

AMERICAN MEDICAL ASSOCIATION
535 North Dearborn St. CHICAGO

CONTENTS

PAGE

HEMORRHAGE AND "SHOCK" IN TRAUMATIC ... CHANGES IN TOTAL SOLIDS AND BOUND WATER OF ... MUSCLE. WILLIAM SALISBURY, PH.D., AND EL... HS, M.D., CHICAGO 869

NONSYPHILITIC AORTIC VALVE INSUFFICIENCY. ... ON, M.D., MINNEAPOLIS 889

HOMOLOGOUS LIVERS AS A STIMULUS TO HE... NERATION. FRANK A. MCJUNKIN, M.D., AND HER... BRUHAUS, B.S., CHICAGO 900

ARTHRITIS: REPORT OF TWO CASES. HARCH... NT, M.D.; CARL J. SCHERER, M.D., AND ERNEST ... AH, M.D., PHILADELPHIA 909

TUBERCULOUS LARYNGITIS: REPORT OF ... ARTHUR R. STANT, M.D., AND EDWIN F. HERSCH, ... CAGO 917

EXPERIMENTAL ARTHRITIS AND ARTERIO... ASSOCIATED WITH STREPTOCOCCAL INOCULATIONS. ... BENSON, M.D.; KENNETH G. SMITH, M.D., AND ... SEMENOV, M.D., PHILADELPHIA, ORE. 924

ATHEROMATOSIS OF AND ARTERIOSCLEROSIS ... NDERVEER, M.D., TORONTO, CANADA 941

RAPIDLY FATAL INFECTION TO TUBERCLE BA... COMPARI-SON OF TUBE REACTION IN NORMAL ... CULOUS GUINEA-PIGS AND IN GUINEA-PIGS IMM... WITH DEAD BACILLI, FRANCIS R. LOWE, M.D.; A... VORWALD, PH.D., AND LORAN DONALDSON, M.A., ... 956

GENERAL REVIEWS:

PELLAGRA: ITS ETIOLOGY (MODERN THEORI... PATHOLOGIC ANALYSIS, HARVEY S. THATCHER, M.D., ... OCK, ARK. 970

THE ETIOLOGY OF CANCER: I, TISSUE TR... TION AND 983

... AND NEWS 1014

... RECENT LITERATURE 1015

SOCIETY TRANSACTIONS:

...OLOGICAL SOCIETY OF PHILADELPHIA 1045

... REVIEWS 1047

... RECENT 1050

... LIST 1051